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COLUMNS

Fighting the unpleasant side-effects of aging

Impact of Bioactive Collagen Peptides® on joint and bone health

Osteoarthritis and osteoporosis affect millions of elderly people around the world and the numbers are rising. Yet, across the globe, people are counteracting the negative effects of aging by choosing healthier diets. A growing amount of scientific data suggests that collagen peptides can play a key role as part of a whole diet approach to promoting health, increasing longevity and reducing the risks of a wide range of age-related conditions. More specifically, supplementation with GELITA's specific Bioactive Collagen Peptides® could help to maintain mobility and improve overall quality of life.

JOINT HEALTH

With its extensive product range, GELITA offers collagen peptides with confirmed efficacy in various applications. FORTIGEL® has been scientifically demonstrated to measurably stimulate the synthesis of cartilage tissue. Thus, it counteracts the wear and tear on the joint cartilage caused by factors such as aging, excessive weight bearing or extensive physical exercise. Taken orally, these specific collagen peptides effectively combat the progressive degeneration of cartilage tissue. Its considerable advantage compared with other ingredients typically offered to improve joint conditions is that FORTIGEL® treats the actual cause of the problems rather than just the symptoms.

PROVEN EFFICACY

After oral administration, FORTIGEL® collagen peptides pass the mucosa, enter the bloodstream partially in an intact form and subsequently accumulate in the joint cartilage. Once in the cartilage, they stimulate the cartilage cells (chondrocytes) to increase the production of both collagen and proteoglycans — the two major components that make up approximately 90% of cartilage dry mass.

To detect the possible mode of action, a study was conducted with patients with mild osteoarthritis of the knee joint (1). The objective of this investigation was to detect and confirm structural changes in the joint cartilage, which was evaluated using dGEMRIC (a magnetic resonance imaging technique).

Although a progressive loss of cartilage was observed during the 1-year study period in the untreated placebo group, the subjects receiving FORTIGEL® showed significantly reduced degeneration of the extracellular cartilage matrix. As a result, it was shown for the first time in humans that orally administered collagen peptides have a direct influence on cartilage structure (Figure 1).

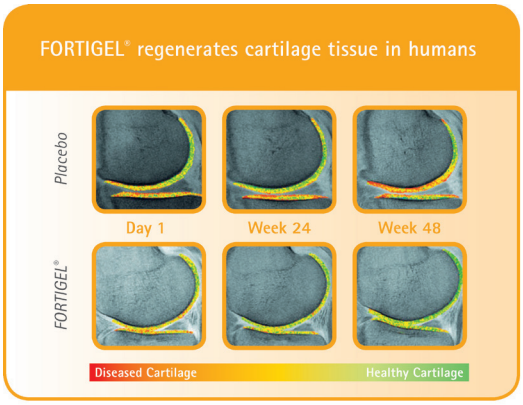


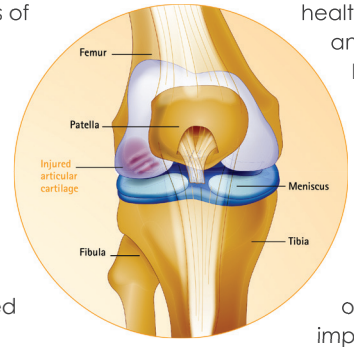
Figure 1. The amount of proteoglycans was statistically and significantly increased in FORTIGEL®-treated osteoarthritic patients when compared with a placebo. The positive effect of Bioactive Collagen Peptides® was clearly visualized using the magnetic resonance imaging technique, dGEMRIC.

To establish a symptomatic improvement in osteoarthritic patients after BCP(Bioactive Collagen Peptides®) treatment, a total of 14 clinical studies have been conducted to date. In the process, more than 2500 individuals have been treated with Bioactive Collagen Peptides®. It can be concluded that collagen peptide supplementation leads to significant pain reduction, a reduced need for analgesics and an improvement of joint mobility.

BONE HEALTH

Besides joint problems such as osteoarthritis, bone-related ailments including atrophy, loss of bone density and stability, and osteoporosis, are also major public health concerns. Based on WHO diagnostic criteria, approximately 22 million women

and 5.5 million men aged between 50 and 84 years of age are estimated to have osteoporosis in the EU (2010 figures). Owing to changes in population demographics, the number of men and women with osteoporosis in the EU is expected to rise from 27.5 million in 2010 to 33.9 million in 2025, corresponding to an increase of 23% (2).



Osteoporosis is a condition characterized by low bone mass and the micro-architectural deterioration of bone tissue that leads to enhanced bone brittleness and an increased risk of fractures. Usually, when we think of healthy bones, we think of calcium; but our bones need more than just calcium. Bone is a mixture of mineral crystals held in an organic collagen matrix. On their own, the minerals would be extremely brittle and prone to breakage. Collagen makes up 95% of the organic bone matrix and is essential for bone health.

Specific collagen peptides have been optimized to support bone health from within. These collagen peptides (FORTIBONE®) stimulate osteoblast activity to increase the production of the extracellular bone matrix, which is the essential framework for calcium mineralization. In addition, they regulate the degenerative processes that affect bones by reducing osteoclast-based protease production. Hence, these ingredients supply the body with the basic components needed for a strong and stable bone structure, supporting overall bone stability and flexibility.

SCIENTIFIC BACKGROUND

Numerous experimental studies have been done that address the impact of collagen peptides on bone density. Investigations have predominantly focused on the age-related metabolic and degenerative processes involved in bone formation and mineralization. Two clinical studies have shown that supplementation with specific collagen peptides led to a statistically significant reduction in the amount of excreted bone collagen breakdown products (compared with a placebo) (3). Collagen peptide intake also statistically and significantly down-regulated serum levels of both bone degradation markers (UPD and UDPD) (Figure 2) (4, 5).

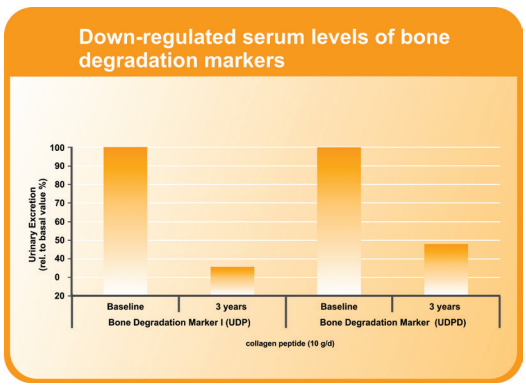


Figure 2. The excretion of bone degradation markers was statistically and significantly reduced after 3 years of oral ingestion of collagen peptides compared with baseline.

The latest study in this field investigated the impact of GELITA's specific collagen peptides FORTIBONE® on postmenopausal healthy women suffering from osteopenia or milder forms of osteoporosis. During this single-center, prospective, randomized, double-blind, placebo-controlled trial, 180 healthy women with a bone mineral density ≤-1 and ≥-2.5 T-value were treated with 5 g of FORTIBONE® daily during a time period of 12 months.

The initial results of this study indicate an anabolic effect — as they revealed a pronounced increase in bone mineral density after FORTIBONE® supplementation in subjects suffering from osteopenia or osteoporosis. In the femoral neck, a significant improvement in bone mineral density (> 6% after one year of BCP treatment) was observed. The bone mineral density in the spine showed a significant improvement of > 5% at the end of the treatment phase. Hence, this new data supports the assumption that FORTIBONE® seem to be an interesting option to counteract bone degeneration and support bone health.

COMPLETELY SAFE WITH NO ADVERSE SIDE-EFFECTS

In addition to their scientifically proven efficiency, Bioactive Collagen Peptides® also have excellent safety profiles. They are exceedingly well tolerated and no adverse reactions have been noted. As FORTIGEL® and FORTIBONE® comprise various short-chain linear peptides, their allergenic potential is extremely low. As they are hydrolyzed to a certain extent, collagen peptides are readily absorbed by the body, easily digestible and highly bioavailable. Additionally, they are free from fat, cholesterol, carbohydrates and gluten, and do not contain purines. Collagen peptides are completely safe, non-allergenic and free from E-numbers, so are perfect for the development of clean label products.

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Crossover between the sports nutrition and health oriented nutraceutical markets

With sales of more than $60 billion in 2016, sports nutrition, including energy drinks, represents the biggest slice of the overall nutraceutical market. The desire for optimized fitness and performance is continuing to grow and products are gaining popularity also with non-athletes. Therefore this nutraceutical segment is predicted to have the highest growth in the coming years (1).
In addition to carbohydrates and vitamins, the effects of protein and essential amino acids including branched chain amino acids (BCAA) on sports performance enhancement are well understood and positively perceived by the consumer. The main idea is to supply the natural building blocks that are limiting muscle biosynthesis and energy generation. In the case of protein, amino acids and their metabolites, there is a straightforward biochemical explanation for the effects that can often be observed in a relatively short timeframe and are well documented in a multitude of studies. Not surprisingly, these proven effects on performance and fitness are driving the adoption of some of these products by recreational/lifestyle users. With steadily increasing life expectancy and consequently a higher percentage of elderly people in the population, these products will also play an increasingly important role in attenuation or prevention of age related syndromes like sarcopenia, which is characterized by loss of muscle mass and quality. Maintenance of optimum health is also the desired result for most other nutraceutical applications in addition to sports nutrition. Therefore a physiological rationale for short term performance or anabolic benefits of "classical" health ingredients in sports nutrition is not always straightforward.
So which are these health ingredients that are moving into the sports nutrition arena and what is the scientific evidence supporting their beneficial effects? In this context, two interesting groups of ingredients are polyphenols and omega-3 fatty acids, which are among the most successful nutraceuticals with proven health benefits and approved EU health claims. Both omega-3 fatty acids and polyphenols can improve cardiovascular health, which obviously provides long term benefits regarding sports performance. In addition to this, there is increasing evidence that they can provide short term benefits in sports nutrition.
A recent meta-review comes to the conclusion that there is a positive effect of polyphenol supplementation on endurance (2). The authors mention several hypotheses that might explain these observations, including upregulation of mitochondrial biogenesis, vasodilatory effects due to increased endothelial nitric oxide synthesis and increased fat oxidation. However, the overall number of studies is still limited and differences between polyphenol subgroups as well as dose-response effects are not well understood.
Supplementation with omega-3 fatty acids in sports nutrition has been shown to have beneficial effects on muscle protein synthesis and metabolic flexibility as well as neuromuscular function. Positive effects on endurance performance, immune function and oxidative stress are so far less convincing. A recent review on this topic comes to the conclusion that while certain groups of athletes might benefit from supplementation and there is a number of studies showing promising effects, there are also many studies that did not show improvements and additional studies are required to come to a clear and general recommendation for omega-3 fatty acids supplementation in sports nutrition (3).
The conclusions from these reviews are not surprising, considering the underlying complexity and number of parameters that have to be controlled. Differences in administered doses, experimental protocols and read out can contribute to variations in the outcome. In addition, the group of polyphenols comprises many chemical compounds and the composition of omega-3 fatty acids can also differ depending on the source and processing of the material. This makes comparison between studies and general conclusions naturally more difficult. Finally, it is well known that formulation can also affect the bioavailability of these compounds. These factors should be clearly defined in future studies to come to a better understanding and clear recommendations for supplementation.
In conclusion, there is a sustained trend of sports nutritional concepts being adopted by other consumer groups but there is also increasing interest in investigating the effects of established health ingredients in sports nutrition. This means plenty of opportunities for science-based product innovation. It will be interesting to see how this develops.

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*Dr. **Martin Schilling** is a Senior Project Manager in the Strategic Innovation Group of Evonik Nutrition & Care GmbH. With a background in food- and biotechnology, he helps to identify innovation opportunities for Evonik's Product Line Pharma & Food ingredients."*

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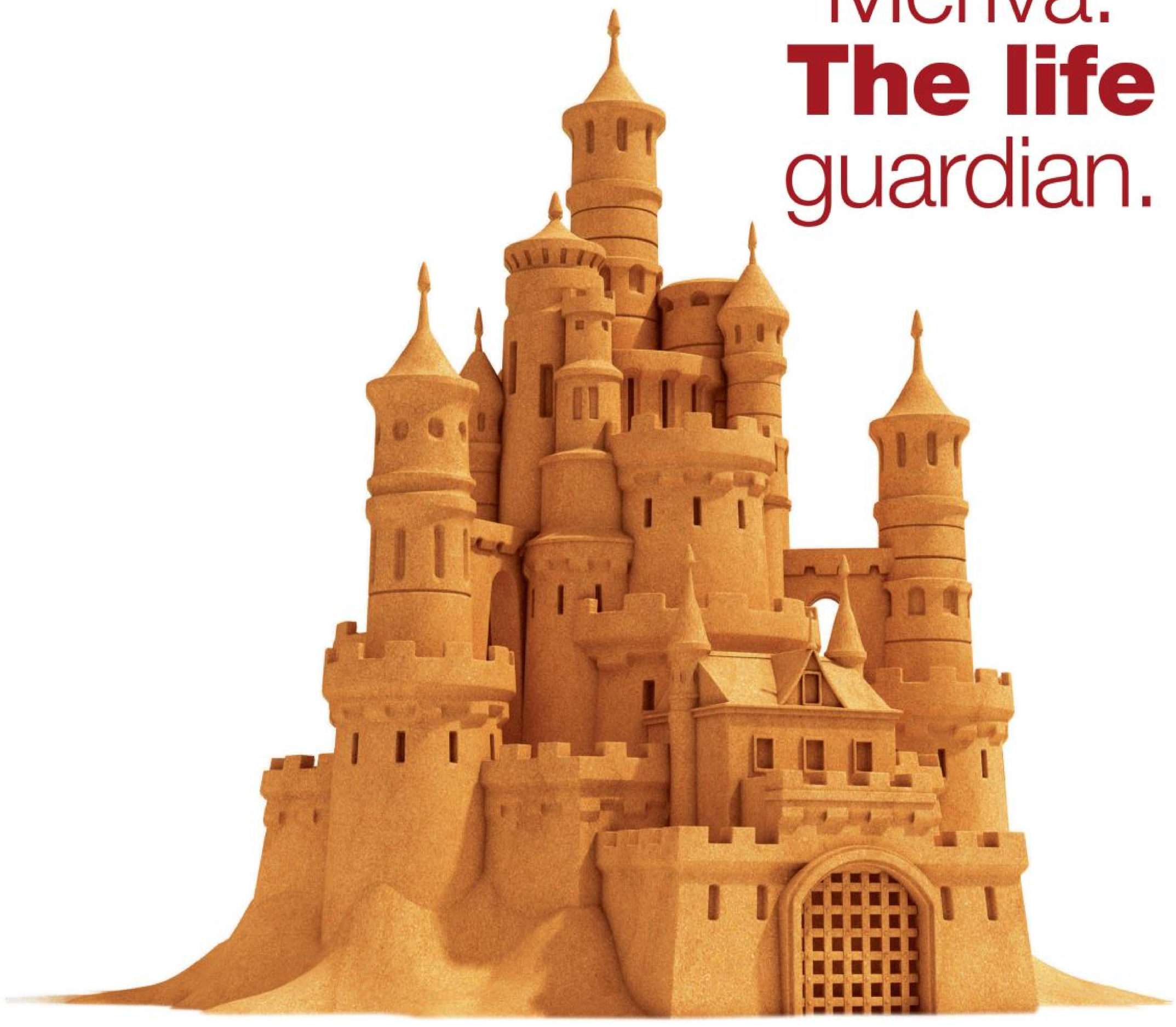

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ADAM CAREY
Chair of the European Specialist Sports Nutrition Alliance (ESSNA), United Kingdom

A healthy market - the rise and rise of sports nutrition

KEYWORDS: Sports nutrition, legislation, European Commission, European Parliament, protein.

Abstract How has sports nutrition moved into the mainstream? After more than a decade of debate around how the industry should be regulated, last year the European Commission confirmed that there need be no specific legislation covering sports nutrition – and like any other food product, the sector will fall under general food law. This is a clear case of the law catching up with real life. Sports nutrition products are much more widespread today than they were 10 years ago, and are used by anyone from a casual jogger to a martial artist. The new legislative framework recognises this, acknowledging that sports nutrition is a safe, effective part of the food industry and liberalising laws covering it accordingly.

The story of sports nutrition is the story of a once niche sector of the food industry that has grown into something widely accepted as mainstream. Witness the sheer volume of products that now come fortified with protein – traditionally a food ingredient most commonly associated with sports supplements. Everything from cereal bars on the shelves next to cornflakes, to drinks on the shelves next to cola, to bread on the shelves next to bagels, can now be found with added protein. Indeed, sale of protein products alone have rocketed 20% in the last five years in the UK (1).

Witness too the types of consumers you see with sports nutrition products: everyday people, such as a mother dropping her child off at school or an office worker on their morning commute, looking to support a healthier, more active lifestyle. Sports nutrition ceased to be the preserve of elite athletes a long time ago, and its use is in no way limited to weight lifting or bodybuilding, as it was traditionally perceived. While it remains a cornerstone in the daily regimes of international sports stars, it is today used by millions of fitness and sport enthusiasts worldwide, and anyone from a marathon runner to a casual swimmer to a martial artist can be seen eating a protein bar or rehydrating with an electrolyte drink. UK consumers alone spent £66m on sports nutrition in 2015 (2).

Furthermore, the sale of these products too has also moved into the mainstream. Sports nutrition products aren't just sold in gyms anymore, but can be found in supermarkets, retailers, pharmacies, health shops, and of course, the internet.

SO HOW DID THE INDUSTRY GET TO WHERE IT IS TODAY?

For all the super-modern drinks, gels, tabs and other products on the market today, the concept of maximising performance in sport and exercise is an ancient one. As early as the Ancient Olympics in 500BC, great athletes recognised the need to support their performance through the intake of nutrients and consumed vast quantities of meat, bread, dried fruits and honey, and various fungi and herbs.

But the modern sports nutrition market is generally agreed to have been established in the 1940s. It was founded on the demands of bodybuilders for ways to improve their performance, which brought about the first generation of sports nutrition products. At the time this meant sweets, starches and sugary drinks – a revolution on the previous use of alcohol, strychnine and other questionable 'supplements' that athletes had mistakenly believed would aid their performance.

The following decades saw rapid evolution. Coaches and their charges not only recognised the benefits of sports supplements but embraced their use. Scientific studies demonstrated the benefits of different ingredients, spawning innovation and the development of complex products unimaginable in the 1940s (3). Further studies and scientific literature demonstrated the increased nutritional needs of athletes that could not be catered for within the daily diet due to a lack of knowledge, lack of convenience or an unwillingness to adopt stringent dietary requirements.

Today, this popularity and acceptance has been driven by convenience, with consumers responding to the availability of nutrition bars, carbohydrate drinks and protein shakes as easy ways to meet their energy and nutrition needs in busy and time-pressured lives. But it's also a story of clever marketing, of people looking to become healthier and more active – a flipside of the obesity epidemic – and taking

advantage of the e-commerce revolution; products like protein powders can be packaged and distributed much more easily now by online retailers.

Much of this comes down to evolving legislation in the European Union over the past years, which has been led by the European Specialist Sports Nutrition Alliance (ESSNA). The legislation of the industry too has had to evolve dramatically in order for the sector to get to the position it's in today.

In 2003 the European Commission wanted to seriously restrict the composition of sports nutrition products by introducing overly strict legislation that would make it impossible for the industry to innovate or change how key ingredients were delivered to consumers and their quantities. This was the reason that ESSNA was formed and it fought hard against the Commission's draft law, engaging extensively with the Commission, MEPs and Member States to emphasise how these rules would negatively impact a small but growing sector, as well as prove a disaster for consumer protection, particularly combined with the growth of e-commerce.

The journey was long and difficult, but in 2011, the Commission published newly proposed laws which instead suggested that, since sports nutrition is not aimed at a particular vulnerable group of people (such as, for example, infant foods), it should be considered a general food. And because there was already a general food law that covered most aspects of selling a food, from labelling to composition, sports nutrition could simply be regulated under that. This was followed by a further few years of intensive engagement to ensure that Member States and MEPs, the two co-decision makers in Europe, did not try and include sports nutrition in specific legislation when the Food for Specific Groups Regulation (EU) No 609/2013 finally became law in 2013 (4).

It took 13 years in total, but the Commission finally released its report in June 2016 confirming once and for all that there was no need for specific regulation of sports nutrition. The debate on how to regulate sports nutrition at an EU level was finally closed, the sector in the EU was given regulatory certainty and the industry was at last free to continue to innovate and develop a whole range of exciting new products.

This isn't to say that challenges don't still remain – there are still several important issues to resolve, both within the industry and with EU authorities. The single market is incomplete and consideration must be given to how sports nutrition will actually function across the different Member States. Setting aside the complications of Brexit, even those countries committed to the EU project can sometimes be slow in implementing all aspects of its single market. Different Member States retain different attitudes to sports nutrition and some of them end up creating significant barriers to trade.

Another main issue is that the sector faces reputational threats through non-compliance. As with every industry, there are rogue companies existing that operate and trade illegally, whether that's by misleading consumers about their ingredients or using false claims about the benefits of their products. ESSNA is working diligently to meet those challenges through engagement with Member States, enforcement bodies, regulators, national anti-doping organisations, and more recently, the general public.

Consumer education is key to consumer safety, and while the latter has always been a priority and at the heart of ESSNA's work, it has only recently become clear that it is very much dependent on the former. Therefore, ESSNA is in the process of launching a large-scale campaign, the aim of which is to ensure consumer safety and good health. The objective is to better educate the sports nutrition consumer, subsequently ensuring that the public are taking products purchased from reputable retailers, that are appropriate for their needs, are in the correct dosages and consumed for the correct reasons, keeping them safe and healthy. Within that, ESSNA will be looking to improve the reputation of the industry and to combat the lingering public and media perceptions about the sector through sharing accurate information and highlighting the significant benefits of sports supplements if taken properly.

So there is still a little way to go and plenty of work for the industry to do to ensure its continued growth and success – but it's safe to say that the sector is unrecognisable today from where it was when ESSNA was founded in 2003.

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Keeping fatigue at bay with L-ornithine

KEEPING FATIGUE AT BAY WITH L-ORNITHINE

The interest in sports nutrition has never been greater. Consumers have learned from elite athletes that performance and recovery can be optimized by choosing the right nutrients. Consequently, sport supplement usage is not limited to professionals. Students, recreational athletes and "weekend warriors" contribute significantly to the growing sports nutrition market.

PROTEIN REMAINS CATEGORY LEADER, BUT THERE IS POTENTIAL FOR INGREDIENTS ALONGSIDE AN EXISTING PROTEIN REGIMEN

Protein and amino acids are among the most famous dietary supplements to enhance strengths, power and muscle recovery associated with training. Indeed, research indicated that individuals engaging in exercise require higher levels of protein for muscle protein synthesis and repair of exercise-induced muscle damage (1). Branched chain amino acids (BCAA), a major building block of muscle protein, are particularly important.
During prolonged exercise, skeletal muscles oxidize BCAAs to meet the increasing energy demand. In this regard, the supplementation of BCAAs can help reduce protein degradation, attenuate glycogen depletion and eventually improve performance (2). However, some intermediates formed in the catabolism of BCAAs such as ammonia may be harmful at high concentrations (3). Several studies reported that the accumulation of ammonia limits the muscle´s energy supply leading to muscle fatigue and a drop in performance (4). Additionally, high ammonia levels can be toxic to the central nervous system and induce central fatigue.

GETTING MORE OUT OF THE TRAINING BY REDUCING THE FATIGUE FACTOR AMMONIA

Elevated ammonia levels owing to intensive exercise or BCCA ingestion can be brought down with a supply of L-ornithine (5).

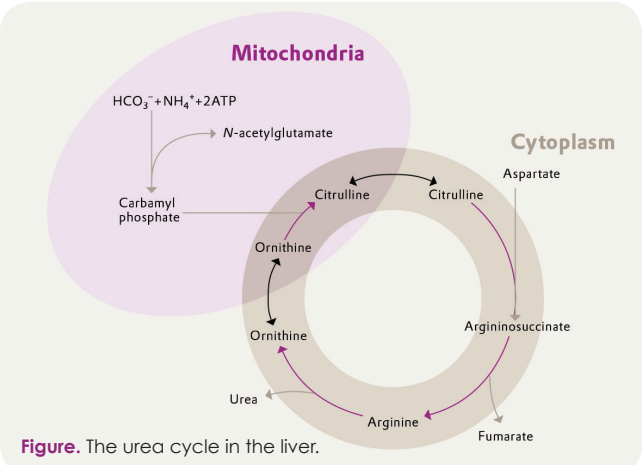

Figure. The urea cycle in the liver.

Being a key component of the urea cycle, L-ornithine promotes a series of biochemical steps, in which ammonia is converted to urea and finally excreted as urine (Figure). Indeed, clinical studies confirmed that taking L-ornithine before training promotes ammonia detoxification (4, 6) resulting in reduced fatigue and improved performance (6). In combination with L-arginine, L-ornithine also increased strength and lean body mass (7).

EVONIK OFFERS L-ORNITHINE SALTS TAILORED TO THE APPLICATION

Based on over six decades of formulation know-how, Evonik has developed water-soluble L-ornithine salts, such as L-ornithine hydrochloride and L-ornithine- L-aspartate allowing for a wide range of sports nutrition applications. Custom-tailored solutions can be offered, as well as finished products such as effervescent tablets, granules and chewable forms of L-ornithine- L-aspartate. In addition to L-ornithine, Evonik supplies other amino acids and derivatives for sports nutrition, including the BCAA (L-leucine, L-isoleucine and L-valine) and L-arginine-α-ketoglutarate, making Evonik the partner of choice for high quality formulations that help stabilize performance, extend training periods and potentially decrease injury susceptibility.

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Registered clinical trials

How science-based are sports nutrition supplement? A PubMed library search to get a first insight

The global sports nutrition market is expected to grow about 8% within the next 5 years to reach USD 45.27 billion by 2022 due to Zion Market Research. Sports nutrition refers to the consumption of nutrients such as vitamins, minerals, supplements and organic substances that comprise of carbohydrates, proteins & fats. In the past, these products were developed for and consumed by athletes and bodybuilders to improve their overall health, performance, and muscle growth. Today, these products are increasingly attracted by lifestyle and recreational users, which explains the projected significant growth in this market category.

All are looking for support to reach sportive results as soon as possible. Increased performance and quick recovery are consumer demands – but is enough scientific data available to enable product developer to select the right ingredients which fulfill these expectations? And even more, do we know the techniques and biomarkers to be used in human studies to test potential sports ingredients? Do we have the right analytical methods to ensure that sports supplements do not contain compounds, which are related to doping?

These questions raised our interest to carry out a literature research about the science related to sports supplements and its focus areas. One of the well-known and most comprehensive literature database for medicine research, including nutrition is the US national library of medicine, Pubmed.gov (https://www.ncbi.nlm.nih.gov/pubmed/) and therefore, this database was used.

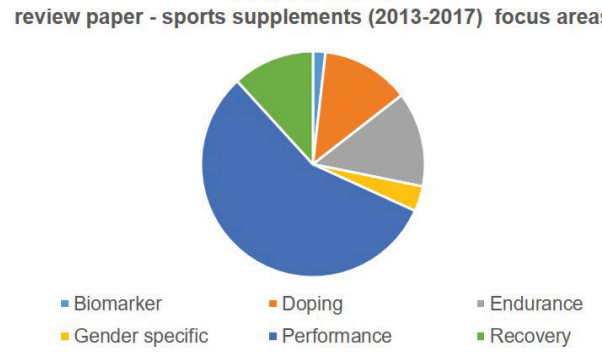

Figure 1. PubMed search: sports supplements review paper (2013-2017) – focus areas.

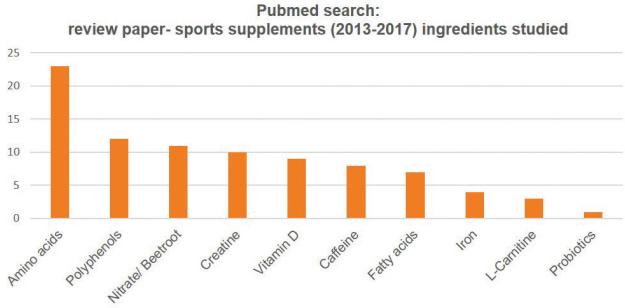

Figure 2. PubMed search: sports supplements review paper (2013-2017) – ingredients studied.

To analyze latest trends and data, it was decided to analyze only review papers about sports supplements which were published within the last five years. 201 publications were found.

The results were evaluated based on 4 criteria. First, the overall focus area of the research within the sports nutrition target area; second, the functional ingredient or ingredient category investigated; third, if the research was related to a specific type of sports; and fourth, if specific techniques or biomarkers were used frequently.

Let's start with the focus areas of the sports supplement studies. More than half of them (56%) focused on performance, followed by studies, particularly looking for endurance as outcome criteria (13.6%) and studies investigating recovery (11.8%). Also, doping related topics were of high interest, represented by 12.7% of the overall publications. In addition, some studies about gender-specific sports nutrition were found (3.6%) and only 1.6% related to Biomarker investigations. Looking at the ingredients being investigated, it was not a surprise that the highest number of studies was found for amino acids (23 studies) followed by polyphenols (12 studies). Nitrates / Beetroot

were also investigated intensively for sports nutrition benefits during the last years, counting 11 studies. Classical sports nutrition ingredients, like L-Carnitine (3 studies), Creatine (10 studies) and Caffeine (9 studies) were found as well as new entries, like Probiotics (1 study) and Vitamin D (8 studies).

Interestingly, most of the studies were not designed to obtain results for a specific type of sports. Only three studies were dedicated for soccer and four for team sports and three for body building.

Techniques or biomarkers which were frequently used, could not be identified. There is not a general study design for an endurance study or a performance study for resistance training effects. This gives a hint, that more basic research is necessary to identify the right parameters for sports nutrition studies and to validate them in repeated trails, so that a guideline for sports nutrition studies can be established. These guidelines would enable industry to carry out studies for potential sports nutrition ingredients in a way, that effects and efficacy can be compared between ingredients and generated data. This would support product developer to choose the right ingredient for the targeted beneficial effect.

This analysis was done only in PubMed and publications of last five years were considered, which means that the evaluation has limitations.
Nevertheless, it reflects that more research in sports nutrition is necessary to fulfill the needs of this growing market category for safe and effective products.
Maybe this would be a good topic for a European project between academia, regulatory authorities and industry. It would be my pleasure to contribute to such an interdisciplinary project.

About the author

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Elite athletes use casein protein for evening recovery

Numerous studies have investigated the importance of protein supplementation in supporting skeletal muscle growth in response to exercise and/or prevention of muscle mass loss during physical inactivity. However to improve the effectiveness of protein, the right protein source and timing of ingestion is important.

TIMING

Apart from protein quality and the amount of leucine, the timing of protein ingestion is also of major important for muscle building and recovery. Per meal or snack, 20 grams of protein maximizes the muscle protein synthesis in healthy young adults (1). Another study confirms that a total daily protein ingestion of 80 grams is more effective when ingested in 4x20 gram instead of 8x10 gram or 2x40 gram (2) portions.

ELITE ATHLETES AND OVERNIGHT RECOVERY

For an athlete, a good night of sleep is crucial. It is possible to boost muscle recovery during the night with the right recovery strategy. Previous studies done in Prof. van Loon's lab have shown that protein ingested prior to sleep is properly digested and absorbed, resulting in muscle protein accretion throughout a night's sleep (3, 4). FrieslandCampina DMV is an expert in milk protein, and is proud to collaborate with the Dutch Olympic Committee (NOC*NSF) and the elite athletes of the Netherlands. It has developed a range of high-protein sports nutrition exclusively for them. Data from the *Dutch Sport Nutrition and Supplement Study* shows that elite athletes consume enough protein during the day but, in the evening, their protein intake is lacking. As a solution, the company has developed a casein protein product that is low in lactose and fat. It provides a slow release of amino acids and supports overnight recovery.
The athletes received the product with enthusiasm. Now, many of them are working with their sports nutritionists to increase their protein intake in the evening to improve their overnight recovery.

STUDY SHOWS CASEIN PROTEIN IMPROVES MUSCLE MASS AND STRENGTH

Whether acute changes in muscle protein synthesis during the night also translate to greater gain in skeletal muscle mass and strength following repeated sessions of resistance type exercise over a prolonged time period, remains to be established. To this end, a study was performed at Maastricht University(5). Forty-four men were randomly assigned to a progressive, 12 week resistance type exercise program. One group received a protein supplement containing 27.5g protein (50/50 Casein and Casein hydrolysate), 15g carbohydrates and 0.1g fat prior to sleep. The other group received a placebo. Dr. Snijders explains why casein is used: 'as casein is known to be a protein that is more slowly digested and absorbed compared with, for example, whey protein, we choose to use casein in the present study to sustain amino acid availability over a prolonged period of time during overnight sleep.'

Figure 1.

After 12 weeks the protein supplemented group showed a significant larger gain in skeletal muscle mass and strength compared to the placebo group (Figure 1). Dr Snijders: 'It is quite remarkable that dietary protein supplementation prior to sleep further increased the gains in muscle mass and strength during prolonged resistance type exercise training in these health young adults, especially

Sjinkie Knegt, gold medalist, member TeamNL

as habitual dietary protein intake was already high in these individuals.' In addition, Dr. Snijders explains: 'The present study confirms our observations that provision of casein protein prior to sleep is well-received and well-tolerated by athletes. From this study we conclude that casein protein ingestion prior to sleep represents an effective dietary strategy to augment skeletal muscle mass and strength gains during prolonged resistance type exercise training in healthy young men.'

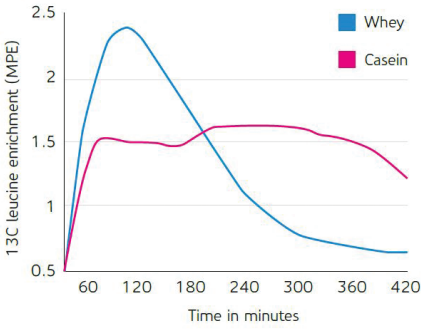

Figure 2. Amount of amino acids released into the bloodstream with casein and whey protein. Casein proteins has a prolonged release of amino acids. Adapted from (6).

CASEIN PROTEIN

In an acidic environment, such as in the stomach, casein protein clots together. The amino acids in casein will be slowly released into the bloodstream6 (see Figure 2). This slow release makes it possible to have amino acids available during a long period (over 7 hours).

FRIESLANDCAMPINA DMV

At FrieslandCampina we take great pride in 140 years of milk processing heritage. As a dairy co-operative, FrieslandCampina has unique control of the complete value chain 'from grass-to-glass'. Together with our strong nutritional know-how we produce high quality nutritional proteins.

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Infant Nutrition

Protein in infant formula: the lower, the better?

Fighting obesity is a major challenge humanity faces today. High protein intake in early life is associated with a greater risk of obesity in later life. Therefore, lowering the amount of protein seems to be the target, but what's the appropiate amount to ensure proper growth? Earlier this year, the European Food Safety Authority (EFSA) published a scientific opinion stating that follow-on formula with a protein content of at least 1.6 g/100 kcal made from either intact cow or goat's milk protein is suitable and safe for healthy infants living in Europe(1). *Although current EU legislation sets the minimum protein content at 1.8 g/100kcal, this seems to be a great step forward towards getting the protein concentration in follow-on formula closer to the levels found in breast milk. However, it is not only the protein quantity that counts, the protein quality is equally important.*

Protein is a key macronutrient especially in early life, because the infant growths and develops rapidly. Deficient protein intake can lead to suboptimal growth and impaired immune- and neurodevelopment of the infant (2). However, protein intake that is too high may lead to rapid weight gain in infancy and is associated with obesity in later life (3). Weight gain from birth to 24 months is the best overall predictor of later life obesity risk (4). Protein concentrations in human milk decrease during lactation from an average of 2.5 g/100 mL (3.8 g/100 kcal) for colostrum (1–5 days); 1.7 g/100 mL (2.6 g/100 kcal) for transitional milk (6–14 days); and 1.3 g/100 mL (2.0 g/100 kcal) for mature human milk from 14 days onwards. The level slowly decreases to about 1.1 g/100 mL (1.6 g/100 kcal) at 6 months of lactation and tends to remain fairly stable thereafter (1).

Protein concentrations in infant formula have decreased over the past decades from 4g/100 kcal in the seventies to minimally 1.8 g/100 kcal nowadays, because a number of human trials have shown that high protein formula consumption leads to a higher obesity risk in later life as has recently been reviewed (5). Current EU legislation sets the minimum protein content at 1.8 g/100kcal, but according to a recent scientific opinion of EFSA, a follow-on formula with a protein content of at least 1.6 g/100 kcal made from either intact cow or goat's milk protein is suitable and safe for healthy infants living in Europe. However, based on available scientific data, EFSA could not establish suitability and safety of infant formulas with a protein content of at least 1.6 g/100 kcal made from either intact cow or goat's milk protein. If this EFSA recommendation is turned into EU Legislation in the future, then future follow-on formulas might decrease their protein content to 1.6 g/100 kcal. EFSA also considered follow-on formulae containing protein from other sources than cow or goats' milk. The available data did not allow EFSA to establish the safety and suitability of follow-on formulas with a similar protein content made from soy protein isolates or protein hydrolysates. Therefore, intactness of the protein and protein source matters. By attempting to mimick the concentration and functional benefits of protein in human milk, it is important to look at protein quantity as well as quality.

The amino acid composition is the most crucial factor in defining food protein quality, protein digestibility, and amino acid availability. Human and cow's milk differ substantially in the ratio of whey to casein protein and in the proportions of specific proteins like alpha-lactalbumin (6). Whey protein and casein are classified as high-quality proteins based on human amino acid requirements, digestibility and their bioavailability.

The amino acid profile of whey protein is similar to that of human milk and superior to that of casein protein. Additionally, whey protein has a superior biological value when compared to casein protein. Whey protein contains a high concentration (26%) of branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine, which are important factors in tissue growth and repair. Leucine plays a central role in protein metabolism during protein synthesis. Whey proteins are also rich in cysteine and methionine; amino acids that enhance immune function through intracellular conversion to glutathione (7). Casein contains a high proportion of essential amino acids such as histidine, methionine, phenylalanine and valine. It also contains a high proportion of non-essential amino acids such as arginine, glutamic acid, proline, tyrosine and serine (8). In order to mimic human milk the closest, regarding the protein quality, the ratio of whey to casein in infant formulas has to be adjusted according to the life stage similar to the protein composition in human milk.
Also alpha-lactalbumin has been used in infant formula to ensure the right amino acid profile for growth as alpha-lactalbumin is the most abundant protein in human milk and contains a high amount of essential amino acid including the rate limiting amino acid tryptophane. Alpha-lactalbumin-enriched infant formulas with a low protein level are clinically documented to support age-appropriate growth and weight gain closer to breastfed infants compared to standard formula (9, 10).
The favourable effect of alpha-lactalbumin supplementation may partly be attributed to a temporarily reduced level of branched-chain amino acids, which may influence weight gain (9). Infant formulas enriched with alpha-lactalbumin and reduced protein level promotes a plasma amino acid profile closer to breastfed infants compared to infants receiving standard infant formulas. Thus, mimicking the protein quality will help in lowering the protein quantity and ensuring appropriate growth and development of in the infant in early life and decreasing the risk of obesity in later life.

KEY MESSAGES

- High protein intake in early life is associated with a greater risk of obesity in later life.
- Protein concentrations in infant formula have decreased over the past decades
- Current EU legislation sets the minimum protein content at 1.8 g/100kcal.
- However, EFSA states that follow-on formula with a protein content of at least 1.6 g/100 kcal made from either intact cow or goat's milk protein is safe and suitable for infants living in Europe.
- To lower protein content in infant formula, protein quality is

as important as protein quantity
- Regarding protein quality, the whey to casein ratio is important as well as proteins like alpha-lactalbumin to ensure the right amino acid profile for growth.

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PEER REVIEWED

Plant-based galacto-oligosaccharides: the new gold standard for special infant formulas?

KEYWORDS: Infant nutrition, infant formula, prebiotic fiber, galacto-oligosaccharides, cow's milk allergy, lactose intolerance.

Abstract

Prebiotics are now considered as key ingredients in infant formulas although their use is not mandatory. The demonstration of their multiple benefits on gut health, immunity and global development in infants has led to the development of several prebiotics among which galacto-oligosaccharides (GOS) and human milk oligosaccharides (HMOs) are now considered as gold standards.

While the prevalence of lactose intolerance and cow's milk allergy are rising in infants and young children, the choice of prebiotic compounds that are safe and suitable for these populations is limited as most prebiotics for infants are produced from dairy raw materials.

A new generation of lactose-free prebiotic substances has emerged with the development of plant-based galacto-oligosaccharides extracted from legumes. Their safety and benefits will be detailed in this article.

INTRODUCTION

The gastrointestinal microbiota of breast-fed babies differs from classic standard formula fed infants: While breast milk is rich in prebiotic oligosaccharides, standard infant formula is not (1).

To deliver benefits as close as possible to those provided by breastmilk, infant formulas manufacturers have added prebiotic oligosaccharides to infant formulas since the late 1990s. Prebiotic fibers addition has since become a key differentiating asset for infant and young children formulas manufacturers. From a physiological point-of-view, prebiotic oligosaccharides addition in formulas modulates positively the microbiota of formula-fed infants and makes it closer to that observed in breastfed infants: lower fecal pH, better stool consistency and frequency as well as more balanced composition of the microbiota (such as higher concentration of bifidobacteria) (1).

Galacto-oligosaccharides (GOS) have established as the prebiotic gold standard in infant formulas as they display molecular features similar to structures found in breastmilk. They have shown their ability to shift positively the microbiota and short-chain fatty acids production in formula-fed infants (2-4) and display a number of potential health benefits such as improvement of stool consistency and frequency (2, 4) and immunity (4, 5).

However all the GOS introduced in the infant nutrition market at the moment are produced from lactose and then cannot be added in formulas targeting infants and young children with lactose intolerance or cow's milk allergy, i.e plant-based and/or hypoallergenic formulas.

While lactose is currently the main raw material used to obtain GOS, legumes are the major sources of GOS in Human diet (6), even though plant-based GOS have seen limited applications on the nutrition market until now. Recently they have been purified and commercialized for different applications including infant formulas where they are aimed at providing a prebiotic alternative to lactose-based GOS in formulas that require being free of lactose or of any dairy ingredients.

The objective of this work was to evaluate the suitability of plant-based GOS as well as their ability to modulate the gut parameters and microbiota in several preclinical models mimicking the infant gut. To do so, plant-based GOS were compared to other oligosaccharides for their prebiotic potential and their ability to prevent the growth of *Clostridium difficile* on an *in vitro* model. Then their suitability and ability to modulate the microbiota *in vivo* was evaluated in neonatal piglets.

MATERIALS AND METHODS

In vitro evaluation of the prebiotic potential of plant-based GOS

Design: Fecal samples from infants aged 0-4 months were collected and co-incubated with different oligosaccharides routinely used in infant formulas. Oligosaccharides tested (6 samples per product) were plant-based GOS (P-GOS®), lactose-based GOS (β-GOS), inulin and 2-fucosyl-lactose (2-FL) that were incubated at 4 mg/mL for 24 hours at 37°C. Additional samples were spiked with *Clostridium difficile*.

Analyses: Fecal DNA isolation was performed by directly transferring 150 mg of fecal material to a DNA isolation plate where 0.5 mL phenol pH 8.0 was added and samples were mechanically disrupted by bead beating then centrifuged. Aqueous phase was collected and purified for microbiota analysis. Analysis of the microbiota composition was performed by mass sequencing of the V4 hypervariable region of the 16S rRNA gene on an Illumina MiSeq sequencer after amplification of the barcoded DNA fragments spanning the Archaeal and Bacterial V4 hypervariable region.

Statistics: Bilateral Student test with significance level (p) set at 0.05.

Safety and prebiotic potential of plant-based GOS in piglets

Design: 2 groups of 12 pre-weaning farm piglets (Yorkshire Crossbred) were fed a pig milk replacer (Solustart® II) without prebiotics (control) or containing 8 g/L of plant-based GOS (P-GOS® P from yellow pea; 90,25 % plant-based GOS) for 3 consecutive weeks. During intervention piglets were observed for growth, feed consumption, moribundity, morbidity and abnormal clinical signs. At the end of intervention piglets were sacrificed and blood and tissues were collected.

Analyses: Cecum and colon contents were harvested and pH was measured. Short-chain fatty acids (acetate, propionate and butyrate) in the colon contents were extracted with diethylether and TBDMSi and measured by GC-MS. Lactate was measured with colorimetric method. Bacterial DNA was isolated from fecal sample by using mechanical and chemical lyses ended by a purification step and total number of bacteria, bifidobacteria and lactobacilli were measured with real-time PCR targeting ad hoc regions of the 16S rDNA. The weight of cecum-colon segment was evaluated.

Statistics: First a Fisher test was applied to determine variance equivalence of both groups' values. Second an unilateral Student test was applied to determine differences between groups. Results of the Fisher test were considered to select the adequate Student test to be applied. Significance level (p) was set at 0.05 for all tests performed.

RESULTS

In vitro effect of plant-based GOS on bifidobacteria and *Clostridium difficile* growth

Plant-based galacto-oligosaccharides increased bifidobacteria levels more than the control, as well as all other oligosaccharides (Figure 1A). The bifidogenic effect of plant-based GOS was similar to that observed with β-GOS and superior to other oligosaccharides (2-FL and inulin).
Growth of the 5 most represented Bifidobacteria species was different across conditions (Table 1). At the species level and compared to control, plant-based GOS increased the growth of *B. longum*, *B. breve*, *B. pseudocatenulatum* and *B. gallicum* but had an inhibitory effect on *B. bifidum*. Compared to other oligosaccharides, plant-based GOS promoted increased growth of *B. longum* compared to 2-FL and β-GOS, increased growth of *B. pseudocatenulatum* compared to inulin, increased growth of *B. breve* compared to 2-FL, increased the growth of *B. gallicum* compared to 2-FL and inulin and decreased *B. bifidum* growth compared to 2-FL and β-GOS.

When co-incubated with a spike of *Clostridium difficile* (Figure 1B), plant-based GOS, β-GOS and 2-FL elicited a similar growth-inhibition effect on the pathogenic microorganism compared to the control, while inulin displayed a lower inhibitory effect.

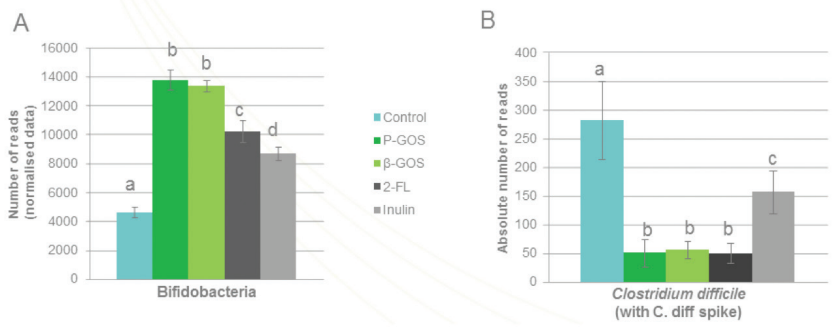

Figure 1. Effect of different oligosaccharides co-cultured with infant stools during 20 hours on bifidobacteria levels (A) and Clostridium difficile levels (after the medium was spiked with C. difficile at t0) (B).

Species \ Conditions	Control	2-FL	β-GOS	Inulin
Bifidobacterium longum	0,000 ↗	0,000 ↗	0,001 ↗	0,850 =
Bifidobacterium pseudocatenulatum	0,000 ↗	0,305 =	0,000 ↘	0,000 ↗
Bifidobacterium breve	0,000 ↗	0,004 ↗	0,060 =	0,004 ↘
Bifidobacterium gallicum	0,000 ↗	0,004 ↗	0,060 =	0,000 ↗
Bifidobacterium bifidum	0,000 ↘	0,043 ↘	0,005 ↘	0,902 =

Table 1. Effect of different oligosaccharides co-cultured with infant stools on the 5 major Bifidobacterium species detected in samples (p-values calculated vs plant-based GOS values; trend shown with arrows for plant-based GOS group vs comparator).

Effect of plant-based GOS on growth and microbiota in pre-weaning piglets

Body weight, body weight gain, food consumption (Figure 2) were similar at all time points in the Plant-based GOS and Control groups, as well as feed efficiency (16,0 vs 15,1 % respectively ; p>0.05 ; data not shown).

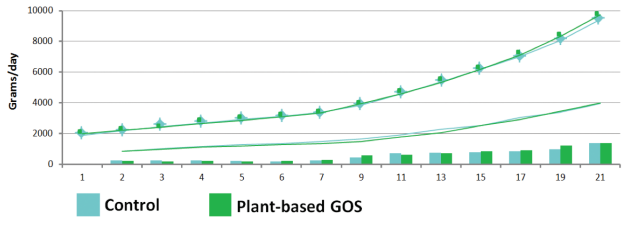

Figure 2. Body weight (dotted line), Body weight gain (bars) and Food consumption (unbroken line) in piglets during intervention ; * p<0,05.

The supplementation of formulas with plant-based GOS resulted in lower fecal pH values in the cecum and colon and increased the weight of the cecum-colon segment (Table 2).

Parameter (unit)	Control (Mean±SEM)	Plant-based GOS (Mean±SEM)	P-value
Total bacteria (nb DNA copies/gram)	$1{,}01.10^{12} \pm 8{,}39.10^{10}$	$1{,}34.10^{12} \pm 1{,}63.10^{11}$	0,045
Cecum pH	6,3 ± 0,1	6,0 ± 0,1	0,030
Colon pH	6,7 ± 0,1	6,3 ± 0,1	0,011
Cecum-colon weight (grams)	119,3 ± 7,9	143,6 ± 11,3	0,047

Table 2. Gut parameters at the end of the piglet intervention.

Plant-based GOS increased the total count of bacteria in the colon contents (Table 2) as well as bifidobacteria count, while lactobacilli levels were similar to Control (Figure 3A). Levels of acetate and butyrate were increased in the plant-based GOS supplemented piglets while lactate and propionate were not different between groups (Figure 3B).

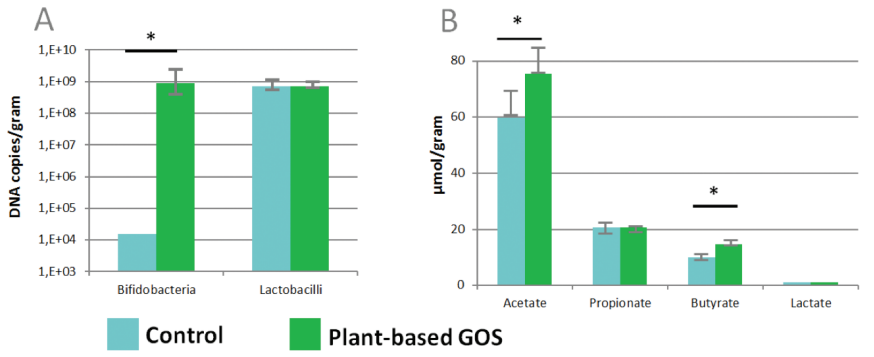

Figure 3. Bifidobacteria and lactobacilli counts in colonic samples (mean ± SEM) at the end of intervention (A) and Acids levels in colonic samples (mean ± SEM) at the end of intervention.

DISCUSSION

Overall our results demonstrate the suitability and nutritional benefits of plant-based GOS in neonates.

The ability to sustain adequate growth is generally considered as a suitability marker when evaluating the interest of new substances introduced in infant formulas (7). In our protocol in neonatal piglets, formula supplemented with plant-based GOS ensured growth patterns similar to those observed with a non-supplemented formula and furthermore improved slightly, despite not significantly, feed efficiency (i.e the ability to extract energy from foods to ensure growth). Additional data published on the effects of plant-based GOS in piglets support the absence of adverse effects in terms of clinical signs, clinical chemistry, hematology, organ weights, or histopathology (8). While rodents are generally the most used animal models to evaluate the safety and suitability of new substances, non-human primates and piglets are considered as more amenable models to mimic infancy conditions as they readily accept infant formulas as nutrient sources (9). Recent position from the European Food Safety Authority (EFSA) clearly supports the use of a repeated dose study in neonatal piglets to evaluate the suitability of new substances, especially when they are not absorbed such as GOS and more generally fibers (10).
The results obtained in piglets support that plant-based GOS display classical features of prebiotic compounds with an acidification of the gut content, the increase in bacterial mass and the increase in the cecum-colon segment weight.

Microbiota results in both experiments support a bifidogenic activity of plant-based GOS similar to β-GOS and superior to inulin and 2-FL. Colonization by bifidobacteria in neonates is considered a key feature of the evolution of the microbiota in early life (11). The genus Bifidobacterium has the ability to process milk oligosaccharides efficiently when milk is the sole source of nutrition. Interestingly while similar bifidogenic effect is observed in plant-based GOS and β-GOS, the specific effect on bifidobacteria shows they each favor specific species, plant-based GOS favoring *B. longum* and β-GOS favoring *B. pseudocatenulatum* and *B. bifidum*, while no difference was observed for *B. breve* and *B. gallicum*. The presence of specific bifidobacteria species in early life has been described in a number of epidemiological studies. *B. longum, B. bifidum* and *B. breve* are present in both formula-fed and breastfed infants in high proportions (12, 13) and have different properties, for example immune-modulating and anti-inflammatory properties for *Bifidobacterium breve* (14, 15). On the contrary *B. pseudocatenulatum* is more representative of an adult-type microbiota (16). Interestingly plant-based GOS have limited effects on lactobacilli populations as observed in the piglet trial, while β-GOS which are made of galactose chains with different linkages generally support the growth of lactobacilli (2).

The pattern of short-chain fatty acids production observed with plant-based GOS shows an increased production of acetate and butyrate. While the exact role of SCFAs in early life is not clearly elucidated, existing evidence suggests that they impact beneficially gut maturation processes (17). More specifically acetate has been proposed as a protective agent against enteropathogens (18) and butyrate as an immune-regulating substance increasing the generation of regulatory T cells (19) which are involved in immune reactions linked to allergies and allergens tolerance acquisition (20). Butyrate has also been described as a potential beneficial compound in the management of infant colic (21) and been shown to relieve visceral sensitivity in adults (22).

The inhibitory effect of plant-based GOS on the growth of *Clostridium difficile* is of particular interest as *C. difficile* is supposed to partly shape the microbiota of infants and is inversely associated with the presence of *B. longum* (23). While the impact of *C. difficile* in the incidence of symptoms such as diarrhea in infants is still debated (24), new evidence suggests that its presence in infancy is linked to allergy development later in childhood (25).

Altogether these findings support the use of plant-based GOS in infant formulas and potential benefits for neonates. In the specific segments of infant formulas aimed at managing cow's milk protein allergy (hypoallergenic formulas) and plant-based formulas they represent a new opportunity to add prebiotics as β-GOS (the current gold standard) and

Human Milk Oligosaccharides (such as 2-FL) cannot be used due to their dairy origin. Furthermore as β-GOS are currently the prebiotic gold standard in regular infant formulas, plant-based GOS could become the new gold standard in hypoallergenic and plant-based infant formulas in a near future. Their use and interest in regular infant formulas requires additional investigation.

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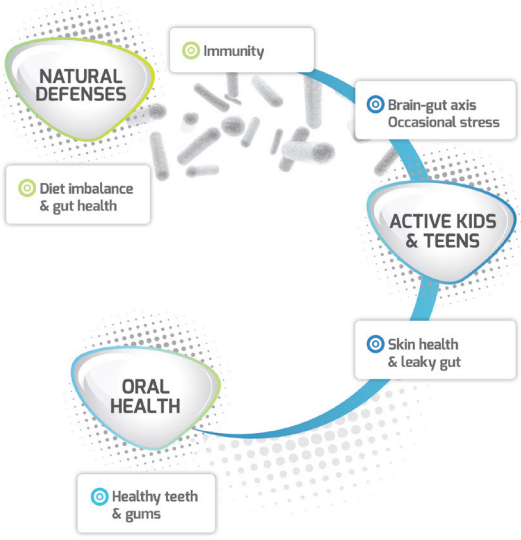
Childhood is a key period for establishment of both the immune system and gut microbiota. **A mature, "adult-like" microbiota is achieved after 3 years old[1], yet kids and teens face many microbiota-related challenges**. According to a WHO survey, 10-30% of 15 years olds girls and boys rate their health as fair or poor[2].

The supplementation of children's diet with probiotics as a natural way to boost their natural defences has become increasingly documented. Beyond the primary, most documented areas for probiotics, namely **gut health** and **immunity**, other key issues in children and teenagers could also be linked to microbiota balance and become potential targets for innovative probiotic solutions, such as occasional **stress, skin** or **oral health**.



BOOSTING KIDS' NATURAL DEFENSES

Gut discomfort is frequent in children and teenagers. This can be linked to poor dietary habits, which in turn greatly affects the gut microbiota composition and balance. More specifically, irritable bowel syndrome (IBS) is increasingly diagnosed in young populations and increases with age. Incidence of IBS-associated abdominal pain in children is 8% to 17% and 13% to 38% in teenagers, with higher prevalence in girls[3]. IBS is associated with daily life discomfort, with impact on health feeling, sleeping behaviour, stress and overall quality of life. It can impact school attendance and success, which in turn generates more stress…

Probiotics solutions

The role of probiotics in preserving and restoring the gut microbiota is well documented, for example:

- Probiotic preparation LACIDOFIL® (*L. helveticus* Rosell®-52 and *L. rhamnosus* Rosell®-11) shows benefits in the **reduction of diarrhea** of various origin, in **lactose intolerance**, or in alleviating **IBS symptoms** (26 clinical studies, including 13 in children)[4].
- *B. lactis* LAFTI® B94 has shown significant improvement of **IBS symptoms** in a study with children and teenagers, without reported side-effects[5].

Immunity matures with age. In the first years of life children are prone to common infections (common cold, ear nose throat, gastro intestinal infections…) as their immune system is building up. This is a particular concern for parents in the first years of socialization (nursery, school), when repeated infections occur, impacting the whole family's quality of life. The gut microbiota represents the first line of defense, forming a protective barrier along the intestinal lumen, but also through several biological mechanisms.

Probiotics solutions

The action of probiotics to improve both the intestinal barrier function and the immune response is increasingly well documented, for example:

- The symbiotic PROBIOKID® (*B. bifidum* Rosell®-71, *B. infantis* Rosell®-33, *L. helveticus* Rosell®-52 and FOS) has been shown to **decrease the risk of occurrence of common infections** in children and limit school absenteeism[6]. Other studies confirm the synbiotic's effects on children's immunity, in particular through positive effect on immunosurveillance[7,8], like increased IgA expression in children with low IgA level. This positive effect could be due to improvement of immune maturity[9].
- *B. bifidum* Rosell®-71 **increased the proportion of healthy days** in students around exam periods (period of increased cold and flu risks)[10].
- *L. helveticus* LAFTI® L10 **reduces the frequency of symptoms** (headache, muscle ache, fatigue and temperature), the occurrence of severe symptoms, and **the use of medication** in students suffering from frequent colds[11].

ACTIVE KIDS AND TEENS

Body's changes in teenagers are associated with new concerns and discomfort.
Stress is growing with age. American survey indicates higher stress level in teens than in adults, especially during the school year, with levels exceeding those perceived as healthy[12]. Over 30% of teens feel pressured by schoolwork[13].
Skin health is associated with hormonal changes in teenagers. There is also potentially a link with gut health and gut microbiota. Acne could well be connected to a leaky gut! It has been shown that 54% of individuals with skin imperfection have significant alterations in the gut flora[14].

Probiotics solutions
Through their interaction with the brain-gut axis, and positive effects on the gut barrier, probiotics represent natural approaches for stressed kids and teens:

- PROBIO'STICK® formula (*L. helveticus* Rosell®-52 and *B. longum* Rosell®-175; PROBIO'STICK®) was demonstrated to improve both gastro-intestinal and psychological symptoms related to stress in healthy but stressed people and promote a healthy mood balance [15,16].
- *B. bifidum* Rosell®-71 shows a reduction of stress-induced diarrhea symptoms and lack of sleep in stressed students[17].
- A selection of strains from Lallemand Health Solutions portfolio can target the leaky gut, promoting the restoration of the intestinal barrier integrity (e.g. LACIDOFIL® preparation, *S. boulardii* yeast) or modulating the inflammation (*B. lactis* LAFTI® B94)

ORAL HEALTH

As kids are growing up, dental hygiene is not always optimal, while their **teeth** capital is being built for life! Under normal conditions, the **oral microbiota** diversity is balanced and microorganisms work together as a natural defence system.

However, this balance can be affected by many factors, linked to kids' lifestyle such as diet and dental hygiene. This can lead to the over-development of certain bacteria involved in dental or gum health problems, such as tooth decay or gingivitis.

Probiotics solutions
In complement to proper oral hygiene, probiotics can contribute to the maintenance of healthy gum and teeth:

- ORALIS SB formulation (combination of *S. boulardii* yeast with *L. rhamnosus* Rosell®-11, *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175) used as mouthwash, significantly reduces the proportion of *S. mutans* a bacteria linked to tooth decay in the saliva[18], and in the dental plaque[19]. It also reduces dental plaque formation, as compared to placebo and to traditional antiseptic mouthrinse[20] and significantly reduced gingivitis risk in kids[21].

IMAGINATION IN FORMULATION

Formulating supplements for children is very specific. Delivery formats, but also choice of flavours and packaging are keys in parents purchasing decision. Lallemand Health Solutions has developed **a wide selection of targeted probiotic formulas and strains with specifically designed delivery forms for kids and teens**, including:

- Powders or orodispersible powders
- Sticks or sachets
- Capsules or easy-to-open sprinkle caps to be diluted in drink (milk, juice) or food
- Chewable tablets

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Safety assessment of additives and ingredients for infants and young children

KEYWORDS: Food ingredients, food additives, infants, exposure, toxicology, safety assessment.

Abstract At the request of the European Commission (EC), the European Food Safety Authority (EFSA) will re evaluate all food additives currently approved for infants and young children. EFSA has subsequently published guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, which details exposure assessment principles and a decision tree approach to risk assessment. A key consideration for the toxicological risk assessment is systemic availability, which for many additives and ingredients may result in expensive and lengthy reproductive and developmental toxicity studies being requested. Exposure assessment becomes more complex for young children (compared with infants) due to an increasingly varied diet, who can have particularly high intakes of specific foods. Experienced scientific judgement is crucial to ensure all aspects of this safety assessment are considered for new and existing food additives and ingredients.

INTRODUCTION

Food ingredients and additives for infants (up to 1 year) and young children (1 to 3 years) require special considerations for their safety assessment. Because risk = hazard x exposure, both must be assessed in this context. Recently the European Food Safety Authority (EFSA) has been mandated by the European Commission (EC) to re-evaluate the safety of specific food additives which are currently, or had been previously, permitted in foods intended for this age group, which will be reviewed in this article.

This review will cover five key areas. First, we will look at the mandates given by the EC to EFSA in relation to the specific re-evaluation of food additives (as well as contact materials, pesticides and contaminants) in foods for these age groups. Second, we will provide an overview of the first stage of the EFSA process – the publication of guidelines for assessing safety for infants less than 16 weeks of age. Third, we will briefly discuss how this new approach may be extrapolated to nutritional/ physiological ingredients being developed and requiring approval (such as novel food ingredients). Following this, other considerations in relation to the risk assessment of ingredients for older infants and young children, mainly related to exposure assessment. will be reviewed. Finally, we will summarise the potential implications for the food industry as EFSA progresses through its re-evaluation program for food additives.

EUROPEAN COMMISSION MANDATES TO EFSA

In November 2014, as a potential result of a new Commission and concerns of the EU member states related to additives to be used in supplements intended for infants and young children, the EC mandated EFSA to re-evaluate those additives currently approved in infant formula, follow-on formula and baby foods. In addition, EFSA was requested to evaluate those additives that had previously been approved in food supplements for these age groups (1).

In its response, EFSA stated that the process would proceed in two stages (2):

1. To re-evaluate the scientific principles of the age threshold and what additional data should be required when assessing safety for this age group – which would be completed by the Scientific Committee of EFSA, its top-level "umbrella" committee. [EFSA had been discussing the threshold for when the normal Acceptable Daily Intake (ADI) applies, which had previously been established at 12 weeks of age by EFSA's predecessor, the Scientific Committee on Food (SCF), and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)]; and
2. The re-assessment of additives currently permitted in the foods intended for infants and young children, followed by those proposed for use in supplements – which would be conducted by EFSA's Scientific Panel on Food Additives and Nutrient Sources Added to Food (ANS).

The EFSA response indicated that the process would be completed by the end of 2019.

In 2016, the original mandate was widened to include pesticide residues, contaminants and food contact material migration. At this stage, the previous age threshold (of 12 weeks) was changed to 16 weeks. EFSA agreed to combine and expand the remit of the request, at least the first stage – the establishment of safety assessment guidance for infants less than 16 weeks (3).

EFSA SCIENTIFIC COMMITTEE GUIDELINES FOR INFANTS UP TO 16 WEEKS OF AGE

Following public consultation, the EFSA Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age was published in the EFSA journal on 31 May 2017 (4). The structure of the guidance broadly covers (a) exposure assessment principles, (b) consideration of the developmental status of the organ systems and (c) the existing toxicity profile of the substances, and suitable animal models that may be more representative for safety studies.

With regard to the exposure assessment component of this guidance, the EFSA Scientific Committee identified a high consumption level of 260 mL/kg body weight/day (from available literature on consumption patterns of breast milk and/or infant formula by young infants). Consequently, when assessing the safety of a substance to be used in foods intended for infants below 16 weeks of age, exposure is determined by multiplying this value by the maximum approved or proposed concentration.

In terms of toxicity testing, the guidance "leads-off" existing guidance established by EFSA in 2012 for the assessment of food additives for the general population (5), which describes a tiered approach (3 Tier system; see Figure 1). The basic battery of toxicology tests required at Tier 1 comprises an *in vitro* assessment of the absorption, distribution, metabolism and excretion (ADME) properties to ascertain whether the compound and/or its metabolites are absorbed from the GI tract and thus "systemically available". Additionally, 3 studies are required at this tier:

- *in vitro* genotoxicity assessment
  - the bacterial reverse mutation test (OECD 471) (6) covering gene mutations; and
  - the *in vitro* mammalian cell micronucleus test (OECD 487) (7) covering chromosome aberrations;
- subchronic toxicity assessment [a 90-day toxicity study performed in rodents (OECD 408) (8), modified to include assessment of endocrine-related parameters].

If the additive is *not* systemically available, is non-genotoxic and shows no evidence of subchronic toxicity in rodents, testing can stop at Tier 1 (with close scrutiny of local effects on the gastrointestinal system in the 90-day study). Progression to subsequent tiers is dependent on specific effects seen in the first tier (see Figure 1).

The new guidance assumes that Tier 1 studies have already been conducted and looks specifically at the immature gut of infants below 16 weeks of age to determine what additional considerations are required. The decision tree is presented in Figure 2.

Essentially, it specifies that if a substance is systemically available (absorbable), an Extended One-Generational Reproductive Toxicity Study (EOGRTS) (OECD 443) (9) is required. This is an expensive and lengthy undertaking. If the additive is demonstrated to be non-absorbable, only a subchronic toxicity study performed in neonatal (young infant) animals is required. Piglet models are mentioned in the guidance as potential models, due to the similarities shared with humans (especially in the gastrointestinal tract).

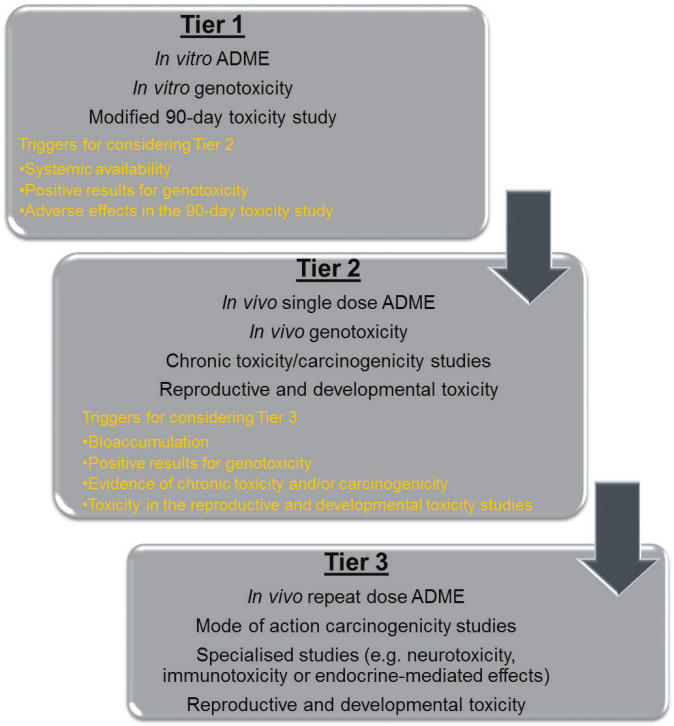

Figure 1. The tiered approach for food additive safety assessment for the general population - *Adapted from EFSA (5)*.

However, there are limitations with using large animals rather than rodents (such as smaller group sizes and less background data); therefore, species selection should be carefully considered on a case-by-case basis.

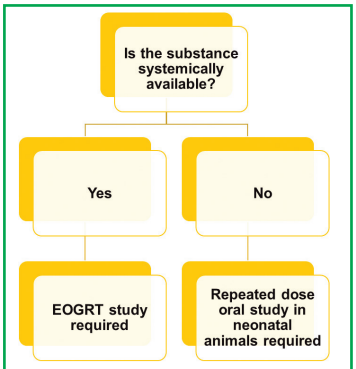

Figure 2. Additional decision tree for infants up to 16 weeks of age.

Recent EFSA Scientific Opinions provide an insight into potential implications for industry. In a recent Scientific Opinion for mixed tocopherols (E 306 to E 309), it was concluded that the re-evaluation was not applicable to infants under 12 weeks of age and that there were insufficient data to address reproductive and developmental toxicity endpoints (10). As a systemically available group of additives, there is the potential for an EOGRTS study to be requested in order to assess their safety for use in foods intended for infants under 16 weeks of age. However, in this case, given their structural similarities with vitamin E (which is widely approved for all age groups), it may be possible to prepare a scientific argument based on safe history of consumption.

One of the few (or even only) additives currently approved for infants which is confirmed as non-absorbable is guar gum (E 412). A recent EFSA Opinion on the general food additive re-evaluation of guar gum stated that "for uses of guar gum in foods intended for infants and young children the occurrence of abdominal discomfort should be monitored and if this effect is observed doses should be identified as a basis for further risk assessment" (11). The Panel concluded that in the absence

of adequate specific studies, it was not possible to assess the safety of use of this additive in food categories intended for special medical purposes for infants and young children. The implication here is that EFSA is looking for a tolerability study in neonatal animals to be able to extend its conclusions on safety to infants less than 16 weeks of age.

EXTRAPOLATION OF ADDITIVES GUIDANCE TO NOVEL FOOD INGREDIENTS

Importantly, within the new guideline, EFSA states that the main principles of the toxicological requirements can be read-across (on a case-by case basis) to all types of ingredients and contaminants in foods for this age group; the guidance does reflect current practice for novel foods for infant formula.

A very recent example of a Scientific Opinion published for an absorbable ingredient is synthetic N-acetyl-D-neuraminic acid (NANA) as a novel food (12), primarily for use in infant formula and follow-on formula. The basis of safety included the following statement "an oral toxicity study in rats with the NF, which consisted of an initial in utero and lactational phase that was followed by a subchronic 90-day oral toxicity study in the first generation offspring". This study is not a replacement for the EOGRTS; however, reproductive and developmental toxicity endpoints were assessed as part of this modified 90-day study. It should be noted that although NANA is systemically absorbed, it is naturally present in human milk (i.e. there is a history of safe consumption) and it is largely excreted unchanged in urine, which may have contributed to the decision that Tier 2 reproductive and developmental toxicity studies were not required. This demonstrates that it is possible to deviate from the guidance, provided there is a robust scientific justification.

An example of a Scientific Opinion published for a non-absorbable ingredient is the EFSA Scientific Opinion on the safety of 2'-O-fucosyllactose (a human milk-identical oligosaccharide) as a novel food ingredient (13). The subchronic 90-day study cited in this Opinion was an adapted OECD 408 study (8) conducted with neonatal rats (rather than the standard OECD 408 approach using weaned rats), which essentially followed EFSA's guidance for infants under 16 weeks of age described above.

RISK ASSESSMENT OF INGREDIENTS FOR OLDER INFANTS AND YOUNG CHILDREN - EXPOSURE ASSESSMENT

At its most basic level, exposure to a substance of interest is calculated based on the consumption of the foods/beverages containing the substance multiplied by the level of the substance in those foods/beverages. Infants and young children are typically noted to have the highest exposure level of all age groups in a population due to their relatively higher consumption on a body weight basis. Figure 3 shows the calculation of exposure (expressed on a body weight basis and as a percent of the ADI) for a hypothetical ingredient ('Ingredient ABC'), which is intended to be used at a single use level (10 mg/L) in 4 different food types (i.e., infant/follow-on and young child formulae, and food supplements for infants and young children). The calculations demonstrate that exposure is affected by (1) the volume of the 'food' being consumed, and (2) the body weight of the individual. For

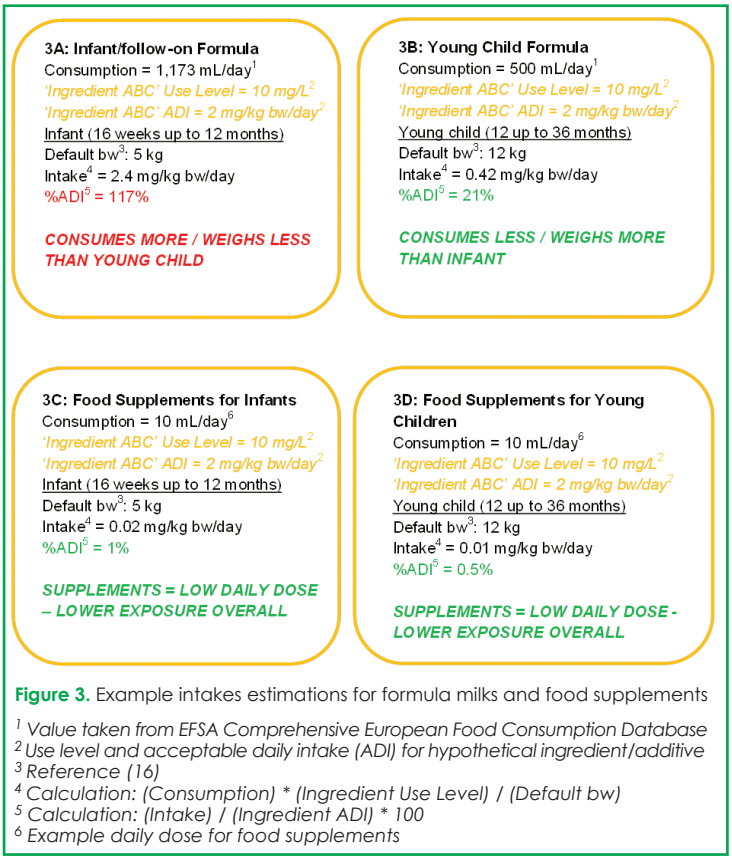

Figure 3. Example intakes estimations for formula milks and food supplements

[1] Value taken from EFSA Comprehensive European Food Consumption Database
[2] Use level and acceptable daily intake (ADI) for hypothetical ingredient/additive
[3] Reference (16)
*[4] Calculation: (Consumption) * (Ingredient Use Level) / (Default bw)*
*[5] Calculation: (Intake) / (Ingredient ADI) * 100*
[6] Example daily dose for food supplements

example, infants (aged 16 weeks to 12 months) consuming ~1 litre of infant/follow-on formula may be more likely to exceed the toxicological threshold of concern than young children (12 to 36 months) consuming formula due to a lower body weight (5 kg versus 12 kg) and a higher level of consumption (1,173 mL/day versus 500 mL/day), see Figures 3A and 3b. In contrast, ingredients used in food supplements will generally have a much lower level of intake than those used in formula, due to the smaller dose of the supplement consumed (~10 mL/day), see Figures 3C and 3D.

While the EFSA guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (4), provided a simplistic approach for assessing high exposure in infants up to 16 weeks of age, this is not necessarily appropriate for older infants and young children (i.e. 16 weeks to 3 years), as this age group have typically commenced weaning, and may therefore be exposed to a greater variety of sources of the substance. Furthermore, this is a cohort associated with unique consumption patterns, such as pickiness and food neophobia, which may lead to higher consumption of particular foods and beverages. In light of these considerations, it is necessary to conduct a more comprehensive assessment of intake for older infants and young children, which considers the total diet.

To this end, there are a variety of resources available to industry to determine potential dietary exposure. For example, the EFSA Food Additive and Ingredients Model (FAIM) template (14) and the EFSA Comprehensive European Food Consumption Database (15), as well as default body weights for different age groups (specifically 5 kg for infants and 12 kg for toddlers) (16), are available. These resources are useful as they allow a simplistic, top-line review of potential exposure to be performed.

However, they can result in large overestimations of exposure, which may indicate safety issues where none exist. Depending on the results from these simplistic tools, it is often necessary to use more sophisticated datasets which are based on actual food consumption patterns by individuals, such as the UK Diet and Nutrition Survey of Infants and Young children (UK DNSIYC) and French Individual and National Food Consumption Survey (INCA) survey data. It is important to obtain the most accurate exposure levels to consider potential future use of the ingredient.

In short, from an exposure assessment perspective, the main considerations are (a) the specific uses and use levels of the ingredient/additive and (b) the characteristics (consumption patterns and body weight) of the target population. There are various resources available, the use of which should be considered carefully.

IMPLICATIONS FOR INDUSTRY

Having considered the detailed review above, the implications for the food industry are quite evident. It is necessary to consider the totality of the evidence available for the substances to evaluate whether they meet the requirements of EFSA's guidance. If there are any "gaps", it is essential that these can be filled, or that a robust scientific argument is developed to negate their need. In practical terms, it is quite likely that EFSA will be resistant to deviation from the guidance, which will result in new studies being requested, likely funded by groups of companies and requests to EFSA for interim measures while these tests are conducted, without threatening the integrity and stability of the food products by hasty withdrawals. Beyond toxicology testing, exposure assessments are complex for weaning infants and young children, where the total diet must be considered. Overall, theoretical study protocols, web tools and databases are a valuable starting point; however, experienced scientific judgement is essential to ensure the interests of the company are protected, and most importantly, that the safety of this critical age group is protected.

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PEER REVIEWED

Proper nutrition against the health effects of air pollution

KEYWORDS: Air pollution, nutrients, vitamins, polyunsaturated fatty acids, oxidative stress, inflammation.

Abstract This article reviews the main constituents of air pollution and their impact on human health. Furthermore, the capabilities and limitations of different defence mechanisms of the human body are discussed. It is emphasised that reduction of air pollution plays the most substantial role in disease prevention, and support of natural defence mechanisms of the human organism should be achieved by antioxidants provided via dietary intake. However, if the antioxidant defence system becomes depleted, complex dietary supplementation should be implemented.

In many cases, the concentration of air pollutants in Europe, especially in cities, exceeds the reference levels that have been established by the EU and WHO (25 and 10 µg/m^3 annual mean, respectively). Among the different types of environmental pollution, industrial and agricultural emissions, vehicles, household heating and cooking are the main sources. According to WHO, around 7 million people die annually worldwide due to diseases caused by air pollution. The main causes of death are lung and heart diseases (1-4). The reduction of particulate matter (PM2.5) concentration to the safety level recommended by WHO (10 µg/m³) would increase life expectancy in the long term by approximately 20 months. Previous studies revealed that the health damage due to air pollution is mainly the effect of oxidative stress, which is the result of the imbalance between pro- and antioxidant processes. This may be caused by a strong oxidant impact on the organism, but it may also be the consequence of the fact that the organism does not have appropriate antioxidant protection (5). Certain nutrients (vitamins C and E, carotenoids, N-3 polyunsaturated fatty acids, selenium, flavonoids) in food serve as antioxidative defence for the organism. The recognition, that air pollutants and some nutrients may interact with one another has increasingly drawn attention to the analysis of the combined impact of these two factors on human health.

AIR POLLUTANTS CAUSE OXIDATIVE STRESS AND INFLAMMATION

Air pollution is a complex mixture of different gases and particles, including the main pollutants causing health problems, namely ozone, nitrogen dioxide and particulate matter (6-7). The latter refers to chemical and physical substances of various origin. Particles with a diameter smaller than 2.5 µm (PM2.5) can reach even the smallest lung alveoli and are always dangerous to health, whereas larger ones (PM10) are normally eliminated by respiration and saliva. Those air pollutants which enter the alveoli of the lungs face a complex biochemical defence system. When the protective capabilities of this system become depleted, the increased oxidative stress may lead to health damage. Free radicals (Reactive Oxygen Species, ROS) are oxidation products generated in the lungs as an effect of air pollutants. Excess ROS production results in oxidative stress, which causes damage in the lipid, nucleic acid and protein fractions of the cells, and consequential tissue damage as well as inflammatory processes are triggered (5, 8). Initially, inflammation serves as a defence mechanism, during which the elimination of harmful antigens occurs, while the formation of ROS continues. At this stage, the induction of antioxidant and detoxifying enzymes (catalase, superoxide dismutase, glutathione transferase etc.) begins, moreover, non-enzymatic antioxidants (reduced glutathione, vitamins C and E, uric acid etc.) also enter the process. These in combination constitute a defensive barrier against further ROS formation in the fluid covering the surface of the respiratory system. In the next phase, if the defence system becomes depleted, the formation of ROS prevails against antioxidants, inflammation accelerates and additional cell damage occurs. Cytokines, chemokines and adhesive molecules generated further facilitate these processes, and the outcome may manifest itself among others in pulmonary and cardiovascular diseases (Figure 1) (5).

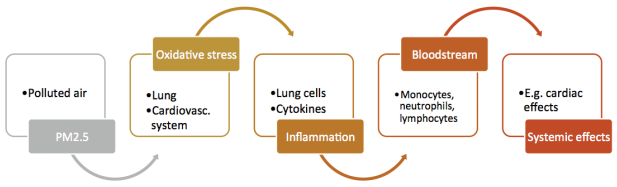

Figure 1. Possible mechanism of action of fine particulate matter (PM2.5) exposure.

VARIOUS ORGAN SYSTEMS CAN BE DAMAGED BY AIR POLLUTION

There is a linear association between air pollution concentration and health risk, without detectable threshold for the latter. Since there is no safe air pollution level at which adverse health effects are absent, national ambient standards do not aim for a zero risk, rather an acceptable one. The health risk of a person can be derived from the combination of multiple exposures and various individual susceptibilities. Numerous studies show that air pollution in high concentrations clearly damages the respiratory tracts. Furthermore, there is evidence that in the long term it is dangerous also in smaller concentrations. Nasal and pharyngeal irritation, and subsequently bronchoconstriction and dyspnoea (especially in asthma patients) are the consequences of sulphur dioxide, nitrogen monoxide, nickel and vanadium exposure. Nitrogen oxides also increase the risk of infections in the respiratory system. In the long term, air pollution can substantially contribute to the development of asthma, emphysema and lung cancer. Small particulate matter induces systemic inflammation and triggers platelet aggregation. As an effect of the latter, due to the occlusion of blood vessels, angina or myocardial infarction may occur. The nervous system is primarily affected by heavy metals (Pb, Hg, As), potentially leading to memory problems, sleep disorders and restlessness. Lead and mercury exposure contributes to the development of certain types of cancer. Heavy metals can also cause reduction in glomerular filtration rate, thus increasing the risk of nephrocalcinosis. Air pollution may have an impact on pregnancy by damaging the development of the foetus, e.g. lead exposure may result in spontaneous abortion, and infants may be born with low body weight. Malformations and disruption of the motor and cognitive functions may also occur (9, 10). Figure 2. summarizes the health effects associated with fine particulate matter exposure.

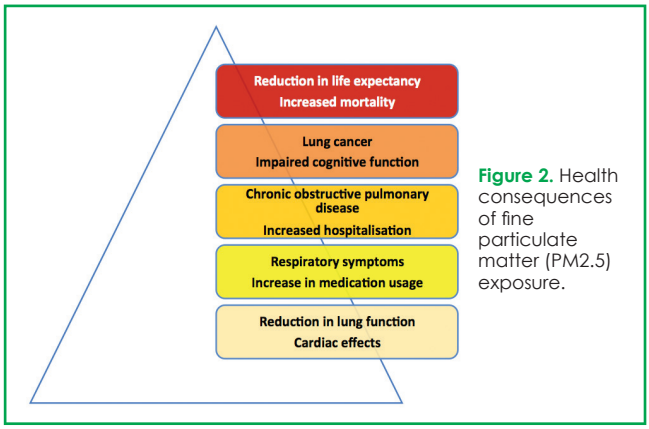

Figure 2. Health consequences of fine particulate matter (PM2.5) exposure.

ANTIOXIDANT NUTRIENTS CONTRIBUTE TO THE BODY'S DEFENCE

Ascorbic acid (vitamin C), a water-soluble vitamin, is an excellent reducing agent and scavenger of free radicals. It provides protection against the damage caused by oxidants both intracellularly and extracellularly. Vitamin C plays an important role in the prevention of lipid peroxidation and in the regeneration of the membrane-

bound oxidized vitamin E (5). Tocopherols (vitamin E) are fat soluble compounds. Due to its effective peroxyl radical scavenging properties, vitamin E prevents the propagation of free radicals in membranes, plasma lipoproteins and polyunsaturated fatty acids (PUFA), thus breaking the chain reaction of lipid peroxidation. Ascorbic acid or other hydrogen donors reduce tocopherol radicals, hence vitamin E can be reused (11, 12). β-carotene (the precursor of vitamin A) and other carotenoids accumulate in the cell membrane and directly react with the peroxyl free radicals generated by some air pollutants, e.g. ozone. They possess antioxidative properties, and regulate inflammatory and immune processes. Other antioxidants, such as flavonoids, are scavengers of superoxide anions and peroxyl radicals. Selenium, an essential trace element, neutralizes peroxides and free radicals (5). PUFAs consist of n-6 and n-3 fatty acids: Arachidonic acid (AA), which belongs to the n-6 family, along with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both belonging to the n-3 family, bear physiological significance. AA, EPA and DHA integrate into the phospholipids of cell membranes, and maintain the integrity, functionality and fluidity of the membranes and affect blood viscosity. By increasing the intake of n-3 PUFA, it is possible to reduce the risk of thrombosis and myocardial infarction. EPA and DHA decrease the growth of atherosclerotic plaques, improve endothelial function in the blood vessels, and reduce blood pressure and inflammation (5, 13). Other micronutrients, like zinc, vitamin A and folic acid contribute to the improvement of immune functions. Several studies show that insufficient supply of these micronutrients significantly reduces the phagocytic capacity of the macrophages, and also decreases the neutrophil mobility and the defence against microbial infections (5).

NUTRITIONAL SOLUTIONS MAY HELP TO COUNTERACT THE HEALTH EFFECTS OF AIR POLLUTION

The oxidative stress caused by air pollution reduces the antioxidant defence capability of the human organism. Thus, accumulation of ROS may be the cause of several diseases due to cell damage and subsequent cell death. The human organism's own antioxidants along with those from dietary intake (primarily vegetables and fruit) may prevent or delay these harmful processes (14). In such cases, when endogenous antioxidants cannot ensure the protection of different tissues and organs, dietary supplementation may prevent the detrimental effects of oxidative stress. However, various studies investigating supplement intake for this purpose do not have a conclusive result (15). A low vitamin C, vitamin E, carotenoid and selenium status has been demonstrated in asthma patients, but the efficacy of the administration of individual nutritional compounds is controversial. On the other hand, anti-inflammatory dietary intervention successfully reduced the risk of asthma exacerbations (16). This reinforces the assumption that a combination of different antioxidant nutrients may lead to the desired health benefits. Several human randomized controlled trials showed positive effects against the health consequences of air pollution, by administering e.g. folic acid (2.5 mg/day), vitamin B6 (50 mg/day), vitamin B12 (1 mg/day), vitamin C (500 mg/day), vitamin E (800 mg/day) and fish oil (2 g/day), among other nutrients (17). Therefore, the use of a combination supplement may be appropriate if the body's antioxidant protection is deteriorated due to certain conditions or if it becomes depleted as a result of extended air pollution exposure.

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Development of a high-protein, plant-based beverage

by using an innovative combination of mashing and fermentation

KEYWORDS: Plant-based beverage, high-protein, product development, fermentation, barley malt, lupin protein.

Abstract The market segment of plant-based foods and beverages is growing rapidly. Combining new food ingredients with existing technology offers great potential for new product development. The aim of this study was to develop a new plant-based beverage with a high protein content, high nutritional value and a refreshing character by a combined process of barley mashing and lactic fermentation. Isolation of protein from *Lupinus angustifolius* as the main protein source was adjusted to obtain a protein ingredient that is soluble in low pH-conditions. Processing conditions during barley mashing and lactic acid fermentation were optimized to reduce antinutritives such as phytic acid and oligosaccharides as well as to improve the flavour and texture of the beverage.

INTRODUCTION

Proteins are an essential component in the human diet, and in particular animal proteins constitute a major protein source all over the world. However, inefficient conversion of plant protein into animal protein raised public awareness in terms of sustainable protein supply in the future. At the same time there is a raising demand for high-protein foods and beverages due to an increase in the consumers' health awareness. Compared to recent developments regarding plant protein alternatives, only few studies have been published focusing on high protein beverages (1-3). So far, most beverage developments are either soft drinks with refreshing characteristics but without protein, or sport drinks based on dairy or soy protein offering high-protein content but lacking a refreshing character. However, plant-based beverages with added protein as well as a refreshing character have not been available up to now. One major challenge is that most plant proteins cannot be dissolved homogenously in acid milieu (4).

Protein isolates from lupin are very promising because they are nutritionally favourable, can be cultivated without genetic modification in Europe and the occurrence of lupin allergy is rather low compared to soy or peanut (5). In addition, sweet lupin contains the globulin fraction *conglutin γ* which is soluble at low pH-values and exhibits desirable techno-functional properties like emulsifying capacity, foam activity and foam stability (4, 6). Nevertheless, this protein fraction can possess disadvantages such as the presence of antinutritive compounds (phytic acid and oligosaccharides) which are typical for legumes, along with a beany and green flavour (7).

Phytic acid acts as an antinutrient due to its complex forming ability with minerals, vitamins and proteins (8, 9). A reduction can be achieved by an enzymatic degradation. According to literature, lactic acid bacteria commonly display high phytase activity (10, 11). Similar, some oligosaccharides in legumes (especially raffinose family oligosaccharides, RFO) are regarded as antinutritives because they cannot be digested by humans due to a lack of α-galactosidase (12, 13). However, it was reported that many lactic acid bacteria are capable of metabolizing RFOs (14-16).

The objective of the present research project was to identify processing strategies for the development of a protein-rich soft drink based on sweet lupines. This required the assessment of an innovative 3-stage process combining protein extraction, mashing and fermentation. An acid-soluble protein fraction, barley malt as a natural source of carbohydrates, enzymes, and flavours as well as microorganisms with high phytase activity were used in order to obtain a nutritionally valuable, protein rich soft drink with pleasant sensory properties.

MATERIALS AND METHODS

Materials

Lupin seeds of the cultivar *Lupinus angustifolius* cv. *Boregine* were provided by ProLupin GmbH (Grimmen, Germany). Barley malt and unhopped Pilsner malt extract was obtained from Mich. Weyermann® GmbH & Co. KG (Bamberg, Germany). Fermenting microorganisms were *Lactobacillus* (*L.*) *plantarum* 758, *L. plantarum*

117, *L. plantarum* 254, *L. plantarum* 727, *L. plantarum* 274, *L. plantarum* 1045, *Pediococcus* (*P.*) *pentosaceus* 95, *P. pentosaceus* 94, *L. casei* 218, and *L. perolens* 532. The strains were isolated from beverages and foods such as Kombucha tea, Kvass beverage, beer, water, Asian sauce and cheese and were provided by the Institute of Brewing and Beverage Technology, Technical University of Munich.

Protein extraction

For extraction of the lupin protein fraction *conglutin* γ ('Lupi F-Extract'), hulled and de-oiled lupin flakes were used. The process was tested in laboratory scale to evaluate the effect of process parameters on protein yield and phytate content. The lupin flakes were extracted aqueously (1:9 w/v) for 45 minutes at 18 °C and pH 4.5. After centrifugation at 9000 g for 5 minutes, the supernatant was filtrated to concentrate the acid-soluble protein fraction. Therefore, an ultrafiltration was conducted with varying temperature (25 and 50 °C), pH-value (pH 4.5 and 7.0) and membrane cut-off size (6 and 10 kDa), respectively (Figure 1).

For extraction in pilot-scale, the most promising parameters were chosen on the basis of the laboratory experiments. The aqueous extraction was conducted at 18 °C and pH 4.5 for 45 minutes. Separation of water-insoluble ingredients was achieved by a separator at 4400 upm. For ultrafiltration, a 10 kDa membrane was used at 50 °C and pH 7.0. The extract was further purified by diafiltration, pasteurization (60 °C, 30 minutes) and spray drying at 185 °C initial temperature, 70 °C final temperature and 1.6 bar (Anhydro Holding A/S, Soeborg, Denmark).

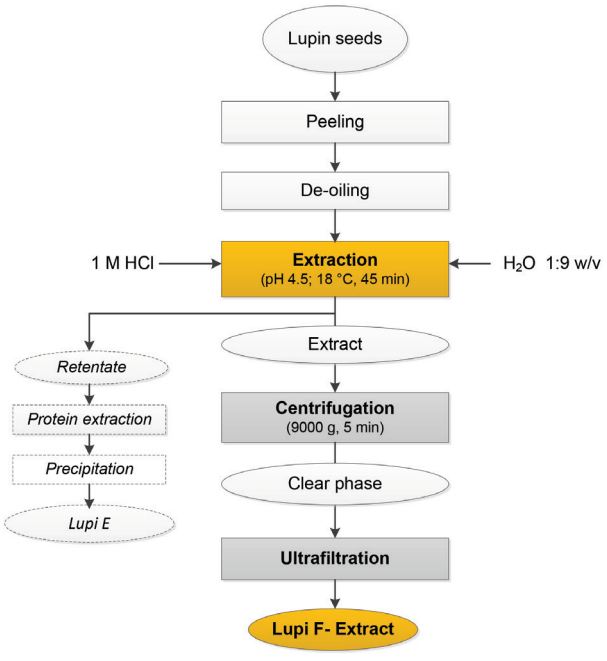

Figure 1. Diagram showing the process for lupin protein extraction.

Mashing procedure

A mashing and fermentation procedure was developed to degrade antinutritive compounds in lupin by means of malt endogenous and bacterial enzymes. Besides, the thermal sensitivity of acid soluble lupin proteins had to be considered. First, barley malt grist was mixed with water (1:8) at 40 °C (Figure 2). After sedimentation of solids, the enzyme rich supernatant was separated and mixed with lupin protein extract. The optimal conditions of phytase were used to degrade phytic acid at 52 °C and pH 5.2. The pH adjustment was performed by the addition of sour malt extract. During the long 'phytase rest' (green dashed line), the residual malt mash was treated at different temperatures (red line) mashing regimes for proteases (52 °C), β-amylase (62 °C), and α-amylase (72 °C), respectively to achieve amino acid and sugar formation. After separation of solids (spent grains) by a lauter tun, the obtained wort was mixed with the enzymatically treated lupin protein. Thereafter, pasteurization was conducted at 62 °C for 15 minutes.

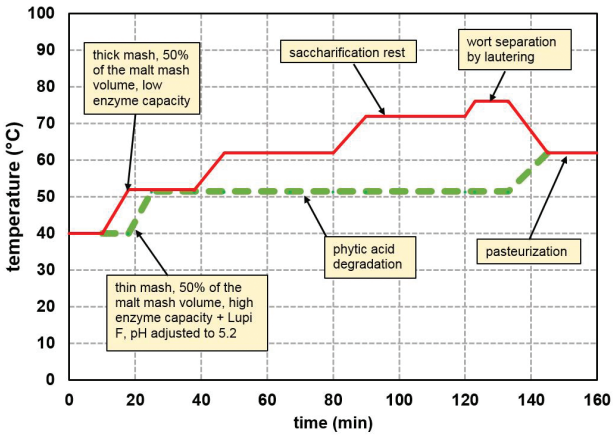

Figure 2. Mashing procedure for barley malt and lupin extract.

Fermentation

Pre-selection of suitable strains

A pre-selection of 26 lactic acid bacterial strains was performed. The main criteria were the ability to reduce phytic acid and oligosaccharides and the formation of a desirable fresh and fruity flavour. For this purpose, a model substrate was used which had a similar composition like the final substrate. It was prepared by mixing diluted (14% w/w) unhopped Pilsner malt extract with lupin protein (1:1). The test fermentations were performed in 100 ml Erlenmeyer flasks at 28 °C with an inoculum of 1 x 10^8 cfu/ml. The high inoculum concentration was necessary to suppress Bacilli spore germination. To examine the aroma profile, the fermented model substrates were evaluated by a trained sensory panel. Furthermore, phytic acid and oligosaccharide reduction were measured as well as the activity of extracellular α-galactosidase.

Final selection

The final selection comprised fermentation trials performed with the strains *P. pentosaceus 95*, *L. plantarum 758 and L. perolens 532* (1 x 10^8 cfu/ml at 35 °C for 48 h) in triplicates. The substrate was prepared by mashing of barley wort with Lupi F as described above. The fermentations were performed in 2 litre flasks.

The parameter settings for scale-up with *P. pentosaceus 95* were 35 °C, 1 x 10^8 cfu/ml inoculum and 24 h fermentation time. The latter was shortened because the results showed no significant differences to 48 h. The scale was raised to 14 litres.

Characterization of fermented drink

Microbial analyses

For examination of the oligosaccharide degradation ability, the concentration of RFOs in the model substrate was measured by the Raffinose/Sucrose/D-Glucose Assay Kit (Megazyme, Wicklow, Ireland). For phytate quantification the phytic acid assay kit (Megazyme) was used. Extracellular α-galactosidase activity was measured using p-Nitrophenyl-α-D-galactopyranoside (Sigma-Aldrich, St. Louis, USA).

Furthermore, a microbial analysis of acid tolerant and beverage typical strains was conducted during storage at 1 °C for eight weeks. For this purpose the selective NBB® and OFS® media (Döhler GmbH, Darmstadt, Germany) were used. The presence of *Bacillus* spp. was analysed by use of HiChrome Bacillus Agar (Sigma-Aldrich).

Nutritional value

Amino acid composition was analysed by Döhler GmbH using EZ:faast kit for GC/FID (Phenomenex, Aschaffenburg, Germany). Vitamin concentration was measured by ADM Wild Europe GmbH & Co. KG (Heidelberg, Germany) using in-house methods and HPLC analysis. The protein content was assayed according to the Dumas method (17) by calculation of the nitrogen content (N x 6.25). The concentration of lactate was analysed by a D-/L-lactic acid Assay Kit (Megazyme) and the amount of diacetyl by headspace capillary GC with electron capture detector. The analysis of fermentable sugars was performed with the Maltose/Sucrose/D-Glucose Assay kit (Megazyme).

Stability test by multiple light scattering

In order to increase cloud stability, emulsions were prepared using coconut oil (Peter Kölln GmbH & Co. KGaA, Germany) in a concentration of 0.25%; 0.5%; 1%; 1.5% and homogenization at 250 and 50 bar (APV-2000, SPX FLOW Inc., Charlotte, USA). The emulsion destabilization was investigated using a Turbiscan Lab® Expert (Formulaction, France) by analysis of transmission (T) and backscattering (BS) profiles as described in literature (18). The emulsions were compared using Turbiscan Stability Index (TSI) that provides information regarding the general behaviour of the samples. TSI is calculated as the sum of all destabilization processes occurring in the sample cell (19, 20).

RESULTS

Characterization of fermented drink

Degradation of oligosaccharides and phytic acid

In a preliminary test, 10 out of 26 examined strains showed α-galactosidase activity (data not shown). These strains were used for fermentation of the model substrate (Figure 3 and 4).

L. perolens 532 degraded most of the oligosaccharides with a reduction of 59%. A significant degradation was also achieved by *L. plantarum* 1045 with a reduction of 53%. The other strains degraded less oligosaccharides but nevertheless a measurable reduction was observed (Figure 3).
The highest phytate degradation during fermentation of

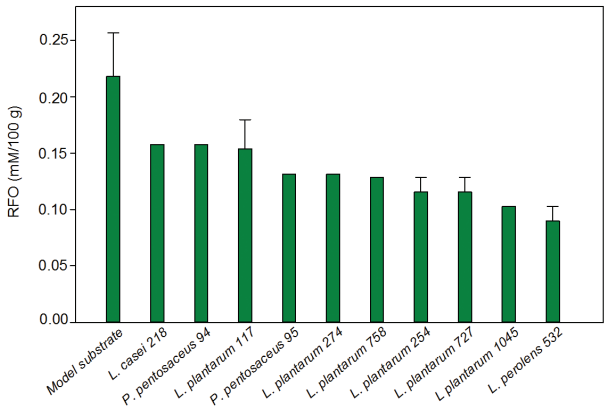

Figure 3. Oligosaccharide content of the unfermented standard and fermented model substrate.

the model substrate was shown by *L. perolens* 532 with a reduction of 22.4% followed by *L. plantarum* 785 with a degradation of 17.5% (Figure 4). *L. casei* 218 and *L. plantarum* 727 showed a decrease around 16%, while the rest of the lactic acid bacterial strain degraded minor phytic acid amounts.

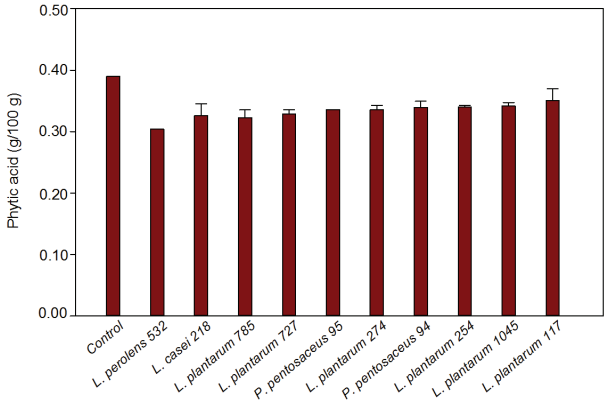

Figure 4. Phytate content of the unfermented and fermented model substrate.

Microbial aroma production

The sensory properties of the fermented products were evaluated by a trained sensory panel from the Institute of Brewing and Beverage Technology (Table 1).

All strains	peach
L. plantarum 758	fruity, less diacetyl
L. plantarum 254	fruity, estery, diacetyl
P. pentosaceus 95	fruity, organic solvents
L. casei 218	fruity, diacetyl
L. plantarum 117	fruity, diacetyl
L. plantarum 727	fat degradation product, musty
L. plantarum 274	organic solvents
L. plantarum 1045	unpleasant, pungent
P. pentosaceus 94	sauerkraut, unfamiliar
L. perolens 532	cheesy, musty

Table 1. Sensory attributes of fermented model substrate.

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The product of *L. plantarum* 758 was rated best due to its fruity aroma without diacetyl or other off-flavours. The fermentation with *L. perolens* 532 was rated worst. Due to the pleasant aroma production, RFO and phytic acid degradation potential as well as further evaluation parameters, *P. pentosaceus* 95 was selected for the development of a high-protein, plant-based beverage.

The final beverage sensory evaluation was characterized by a malty, fruity and citrus-like aroma with a full-bodied mouthfeel. Similar, the odour was described as cereal- and caramel-like, malty, diacetyl and citrus-like. Due to the pH value of 3.6, the beverage has a refreshing sour flavour.

Microbial control

Within the storage period of eight weeks at 1 °C the microbial contamination was below the detection limit (< 1.0 x 10^2 cfu/ml). Therefore, it was ensured that no high cell counts of beverage spoilage bacteria were contained in the protein-rich soft drink. Furthermore, the analysis of *Bacillus* spp. proved no contamination.

Ingredient	Concentration
Phytic acid (g/100g)	0.012 ± 0.001
RFO (mmol/100g)	0.114 ± 0.013
Protein (%)	4.00 ± 0.0
Lactate (g/l)	4.55 ± 0.16
Residual sugar (g/l)	46.1 ± 1.8
Diacetyl (mg/l)	0.033 ± 0.011
Sugar-Acid-Ratio	10.13
pH-value	3.6 ± 0.0
Degree of protein hydrolysis (%)	12.8 ± 0.0
Vitamin B_1 (mg/kg)	<0.2
Vitamin B_2 (mg/kg)	<3.0
Vitamin B_3 (mg/kg)	<3.0
Vitamin B_6 (mg/kg)	<3.0
Vitamin B_7 (µg/kg)	<5.0
Vitamin B_9 (mg/kg)	<1.0
Valine (mg/kg)	121.8
Leucine (mg/kg)	146.9
Isoleucine (mg/kg)	60.3
Threonine (mg/kg)	163.2
Methionine (mg/kg)	46.3
Phenylalanine (mg/kg)	127.2
Lysine (mg/kg)	112.6
Tryptophan (mg/kg)	0.0

Table 2. Analysis of the fermented beverage.

Nutritional value

Table 2 shows the composition of the fermented beverage. The initial phytic acid content of 0.39 g/100 g was decreased during processing to 0.012 g/100 g. Assuming an average phytate consumption of 700 mg phytate per day, which was reported in literature (9, 21), a 500 ml serving of the fermented beverage will contribute to less than 10% to the daily intake. Likewise, the RFO concentration was reduced from 0.21 to 0.11 mmol/100 g, indicating reduced antinutritional properties. The reduction of phytic acid and oligosaccharides can be ascribed to the combination of mashing and fermentation. As described in literature, an eleven fold increase of the phytase activity was measured during germination of barley (22). Likewise, RFO reduction was found during ensiling of legume grains (23).

With a protein content of 4% and a valuable amino acid composition, consumption of the beverage can help to reach the daily recommended level for protein. By uptake of 500 g of the fermented beverage, the daily requirements of the essential amino acids are covered partially for a healthy adult (valine: 8.7%, leucine: 10.5%, isoleucine: 2.2%, threonine: 7.8%, methionine: 2.5%, phenylalanine: 6.5%, lysine: 5.7%, according to Zimmermann M. (24) and WHO, FAO (25)).

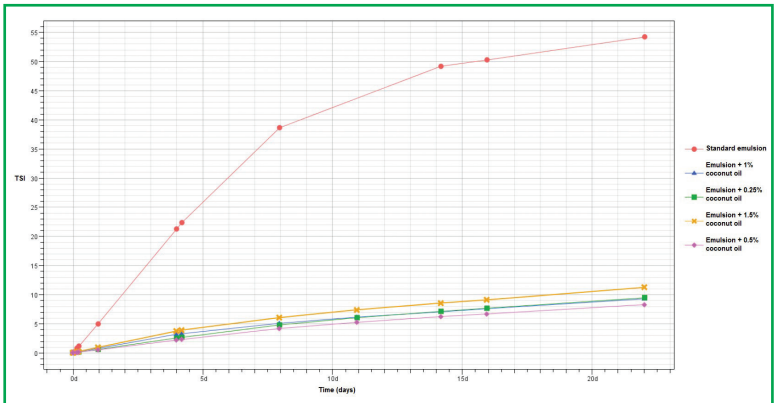

Figure 5. Turbiscan Stability Index of obtained emulsions as a function of time.

Stabilization

The results from stability tests are shown in Figure 5. TSI results enabled a comparison of the stability during storage. The lower the value of the TSI, the more stable was the analysed sample. Emulsification and homogenization increased the cloud stability as indicated by lower TSI values. Out of the lipid concentration tested, 0.5% coconut oil resulted in the best cloud stabilization. The emulsions were homogeneous for 22 days with significantly reduced sedimentation tendency.

CONCLUSION

Recently, plant based proteins are of high interest and are therefore processed in various food applications. This trend is accompanied by the growing demand of consumers for high-protein beverages. Therefore, this project aimed at developing a refreshing and protein rich, plant-based beverage. It was shown that the acid soluble lupin protein fraction is suitable for protein enrichment of acidic beverages. The innovative process combination (mashing and fermentation) of barley malt and lupin proteins led to an attractive beverage base with improved flavour and texture properties. Moreover, the nutritional value was enhanced due to an enzymatic reduction of oligosaccharides and antinutritional phytic acid. The first application tests with different fruit juices were very promising.

Several concepts for fermented protein beverages have been reported recently. Mridula et al. (26) described a non-dairy probiotic drink utilizing sprouted cereals, legume and

soymilk with good sensory properties but a lower protein content of 2.2-2.7 g/100g, depending of the cereal used. A fermented high- protein beverage with 9% protein was developed by Cho, Y.-H., et al. (27), however the drink is not plant based as whey protein concentrate was used as the main protein source.
Against this background, the present study revealed that various attractive plant-based beverages with high protein content can be developed which have a promising potential in the soft drink and brewing industry.

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Raffael Osen *studied food technology at Bonn University. He continued at the Fraunhofer Insitute for Process Engineering and Packaging IVV for his PhD on high moisture extrusion cooking of pea protein. Currently he works as deputy head of the department for food process engineering at Fraunhofer IVV on the development of new food and feed using innovative process technology as well as reformulation of products by utilization of functional ingredients.*

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The market for functional foods, sports nutrition and weight management products continues to boom. While consumers want products with health benefits that taste good and are as natural as possible, manufacturers often face challenges in production. Not all micronutrients and minerals are easy to incorporate into food and drink or are naturally bioavailable.

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IRON SUPPLY HAS MULTIPLE SIDE EFFECTS

Iron deficiency is widespread. Many people – particularly those who follow a vegetarian diet – do not get enough of this essential nutrient, and women of childbearing age often have low blood iron levels. Recent studies have also highlighted the fact that many infants and toddlers do not receive sufficient iron, despite a plentiful supply being particularly important in the first few years of life. Indeed, at all times of life, including during pregnancy, an adequate intake of iron is essential. The consequences of iron deficiency are well known and include fatigue, poor concentration and increased susceptibility to infections.

There are numerous iron-fortified foods and food supplements on the market. However, many of these contain inexpensive sources of the mineral, such as iron sulfate, which has good bioavailability but significant disadvantages. The high reactivity of iron means that iron fortified products oxidize very quickly – they turn an unsightly brown color and "rust" in the truest sense of the word. In terms of taste, there is a negative impact too: the intense metallic flavor of the iron is difficult to mask. A far greater disadvantage, however, is the low tolerability of classic iron products, since the iron ions react in the gastrointestinal tract and often cause irritation or lead to black, very

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To enable products to be enriched with zinc, Taiyo has developed SunActive® Zn. With a 24 percent zinc content, it offers very high bioavailability without an unpleasant taste. Zinc is an essential trace mineral that plays an important role in virtually all of the body's metabolic processes. An adequate supply of zinc is therefore a prerequisite for a strong immune system, for rapid wound healing, for general performance and, last but not least, for fertility. Zinc is also required for mental performance, and recent studies point out that zinc has beneficial effects in the treatment of children with ADHD.

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Coenzyme Q10 is a fat-soluble, vitamin-like nutrient that is normally poorly absorbed by the body but plays a key role in energy production throughout the body's cells. Since coenzyme Q10 is not soluble in water, it cannot be used readily in beverages or food. SunActive® Q10 E, however, is a patented delivery system that allows food and drinks to become effective Q10 suppliers. SunActive® Q10 E makes Q10 easily dispersible in water and increases its bioavailability by 320 percent. Neutral in flavor, with a light yellow to orange color, SunActive® Q10 E looks similar to orange juice. Like the other SunActive® systems, it is heat-resistant, salt-stable and can be used in a wide range of pHs.

Coenzyme Q10 has been extensively researched for more than 50 years and has long been used in the field of sports nutrition. Demand for products that contain it has been growing in the mass consumer market for some years now. In particular, coenzyme Q10 has been shown to improve heart function, promote physical and mental performance, counteract fatigue and lack of energy, and to have a beneficial effect on all bodily functions for which energized cells are important. Not only is coenzyme Q10 popular as a dietary supplement, it is also becoming increasingly sought after in functional foods and beverages. With such widespread appeal, it is important for manufacturers to be able to solve the technological challenges associated with its use in as straightforward a way as possible.

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PEER REVIEWED

Functional hydrocolloids from seaweeds

KEYWORDS: Functional hydrocolloids applications, carrageenan, agar, alginate, seaweed cultivation, West Africa, business opportunities.

Abstract The global production of seaweeds continues to grow for production of food hydrocolloids, i.e. carbohydrate polymers that form viscous suspensions and gels in water. Because of their unique gelling properties seaweed hydrocolloids are used in various food and pharmaceutical applications. Asian countries and Tanzania are currently the main producers of seaweed hydrocolloids based on cultivation of seaweeds such as Kappaphycus alvarezii, Gracilaria spp. and Laminaria spp. that hold carrageenan, agar, and alginate, respectively. In this review we summarize the chemistry, food uses, and gelling mechanisms of carrageenan, agar, and alginate, and describe the key techniques and principles for their extraction from seaweeds. We also discuss the options for local seaweed manufacturing as a business opportunity in countries along the West African coast.

INTRODUCTION

Carrageenan, agar, and alginate are natural polysaccharides that function as hydrocolloids, i.e. substances that interact with water to form viscous suspensions and gels. These hydrocolloids are extracted commercially from certain seaweed (macroalgae) species. Due to their unique gel-forming abilities, the seaweed hydrocolloids are widely used as thickeners, fat replacers, and stabilizers in the food industry and also have numerous applications in biotechnology and in pharmaceutical products e.g. as wound dressings and matrices for control of drug release (1,2). The seaweed hydrocolloids differ in their chemical composition and thus have different gelling properties (ranging from increasing viscosity in solutions to producing very strong gels) and hence different applications. Table 1 highlights some commercial applications of the seaweed hydrocolloids.

Seaweed hydrocolloid	Applications
Carrageenan	Ice Cream (e.g. Ben & Jerry´s (3)), Toothpaste (Colgate (4)), chocolate milk (Nesquik) (5)), Soy milk beverages (6)
Agar	Jelly candy products (Haribo (7)), cultivation media for microbial growth (8), yoghurt (9)
Alginate	Wound dressings (10), Impressions used in dentistry (11), matrices for control of drug release (12)

Table 1. Overview of the seaweed hydrocolloids carrageenan, agar, and alginate, and their applications.

Seaweed derived hydrocolloids offer several advantages over currently used gelling agents, e.g. gelatin obtained from animals and xanthan gum produced by fermentation of carbohydrate sources by *Xanthomonas campestris*, as they do not require land for cultivation, do not interfere with food production, and represent a non-animal derived (suitable for vegetarians), non-GMO natural source of hydrocolloids. The term carrageenan in fact covers a family of different polysaccharide structures having different hydrocolloid properties (discussed below), but carrageenan only has one EU food additive E-number: E407. Agar has E-number E406, and alginate has several E-numbers, E400-E404, depending on composition and which type of cation complex it concerns. The seaweed hydrocolloid industry was estimated to have a value of 1058 million dollars in 2015 and is estimated to grow 2-3 % per year (13). In 2014, the annual global production of seaweeds and other algae for direct consumption or for hydrocolloids production exceeded 28.5 million tons. As the demand for seaweed based products has now surpassed the supply, and the understanding of the structure function relations of these hydrocolloids increases, there is an opportunity to introduce alternative seaweeds and cultivation technologies in more countries to meet the market needs and at the same time create new green growth options and expand the livelihood opportunities for fishermen and women in coastal areas in low and middle income countries (14).

The present paper presents the chemistry, uses, and gelling mechanisms for the three seaweed derived hydrocolloids carrageenan, agar, and alginate along with a description of current cultivation and extraction techniques. The paper also discusses the possibility for local seaweed production along the coast of West African countries.

SEAWEED HYDROCOLLOIDS

Carrageenans are commercially extracted from the red seaweeds *Kappaphycus alvarezii* and *Eucheuma denticulatum*. In 2015, the estimated sale volume of carrageenans was 57,500 tons, with an average price of 9 USD/kg (13). Carrageenans are composed of repeating units of D-galactopyranosyls bound together by alternating α-1,3 and β-1,4 linkages. There are three main commercial

classes of carrageenan: κ-carrageenan, ι-carrageenan, and λ-carrageenan with the primary difference being the occurrence of 3,6-anhydro-galactose and the number and position of sulfate ester groups (-O-SO_3^-) on the repeating galactosyl units, Figure 1 (15).

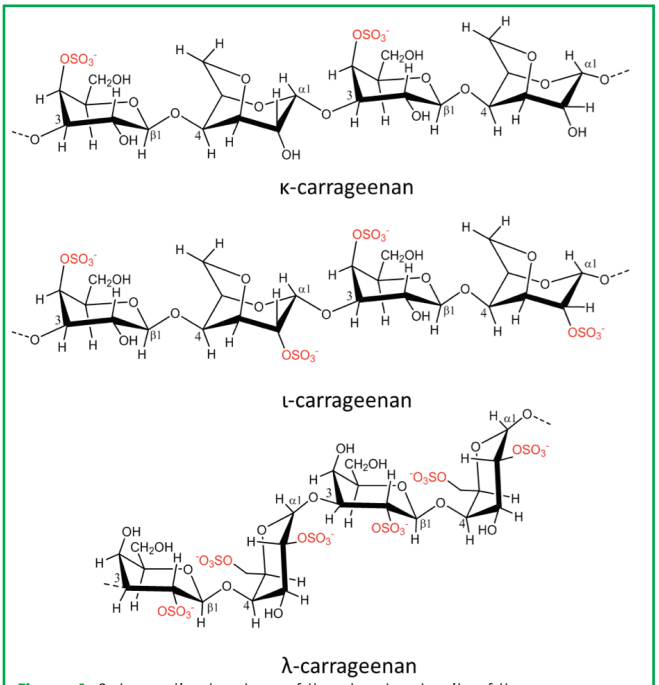

Figure 1. Schematic structure of the structural units of the three commercially available carrageenans: κ-carrageenan, ι-carrageenan, and λ-carrageenan.

The 3,6-anhydro-galactose allows the carrageenans to undergo conformational changes thereby enabling the formation of a gel-network by formation of α-helices. As illustrated below, the presence of cations (potassium for κ-carrageenan and calcium for ι-carrageenan) leads to aggregation of these helices thereby creating a stable three-dimensional network, Figure 2 (16).

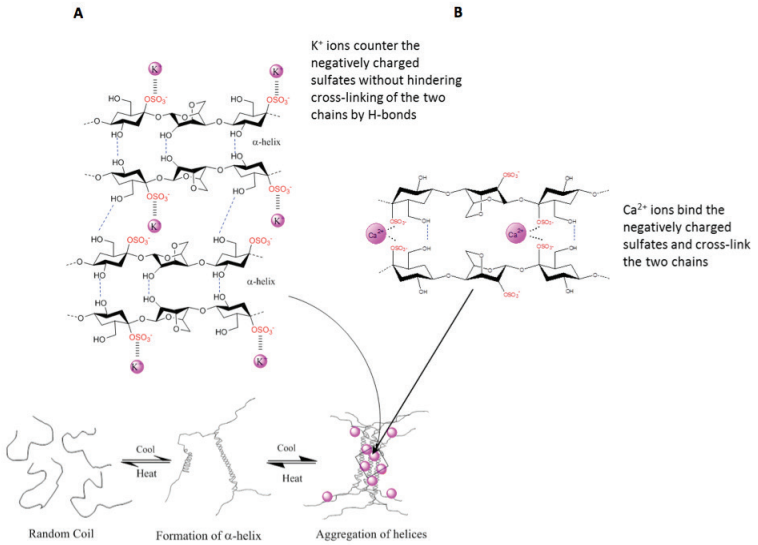

Figure 2. Carrageenan gelation in response to temperature changes and highlighted interactions with different cations. **A:** The gelation mechanism for κ-carrageenan in the presence of potassium ions and **B:** Stylized ι-carrageenan gelation chemistry in the presence of calcium ions (17).

The amount of 3,6-anhydro-galactose and presence of gel-inducing cations thus have a profound effect on the gelling characteristics of the carrageenans: κ-carrageenan produces strong and rigid gels, ι-carrageenan produces soft and elastic gels, and λ-carrageenan does not produce gels (as it has no 3,6-anhydro-galactose) but instead increases the viscosity of solutions (17).

Agar, also extracted from red seaweed sources (Gracilaria spp., Gelidium spp.), is chemically similar to carrageenan by being built of repeating units of galactosyl units bound by alternating α-1,3 and β-1,4 linkages, but whereas the α-linked galactose unit is in the D-configuration in carrageenans, agar contains galactosyl moieties in the L-configuration and is thus made up of α-L-galactopyranosyl units and β-D-galactopyranosyl (15). Agar consists of two types of polysaccharides: agarose (~70%), referring to the so called unmodified part, and agaropectin, which is modified with acidicgalactopyranosyl side-chains such as sulfate and pyruvate (18). Agar gelation principally follows the same pattern as the carrageenans: the 3,6-anhydro-galactosyl units allow the formation of α-helices, though gelation is presumed to take place via hydrogen bonding and without involvement of ions(19,20), Figure 3. The average price of agar was 17 USD/kg in 2015 with a production volume on 14,500 tons (13).

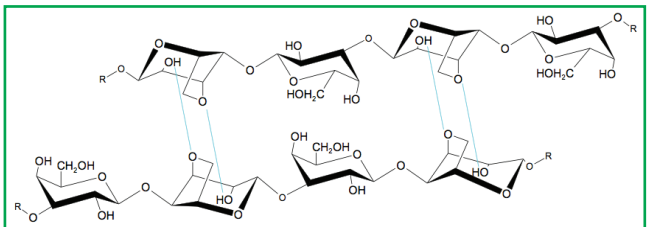

Figure 3. Gelation mechanism of agar. Blue lines represent hydrogen bonds.

Alginate is extracted from brown seaweeds, mainly *Laminaria* spp., and *Macrocystic* spp. The annual global production of alginate from seaweed sources was 26,644 tons in 2015 with an average price of 14 USD/kg (13). Alginate is chemically and structurally completely different from carrageenan and agar. Alginate consists of β-D-mannuronic acid (M) and α-L-guluronic acid (G), Figure 4. The two uronic acids are arranged in a block-wise pattern with varying proportions of 'MM', 'MG', and 'GG' blocks. In the presence of divalent cations, usually calcium, alginates are able to form highly viscous solutions and gels, Figure 4. The gelation process predominantly involves binding of the cations across the 'GG'-blocks of aligned alginate chains, hence the chemical composition, block-structure, and length of the 'MM', 'MG', and 'GG' blocks have a major impact on the physico-chemical properties of alginate. The gelation of alginate follows the "egg-box" model as the divalent cations fit into the structure of the guluronic acid residues like eggs in a box, Figure 4 (21).

EXTRACTION TECHNIQUES

Current extraction techniques for carrageenan and agar are combinations of chemical modification and extraction; enzyme assisted extraction procedures have been reported in the literature (7), but are not yet used commercially. As carrageenan and agar are very heterogeneous carbohydrates,

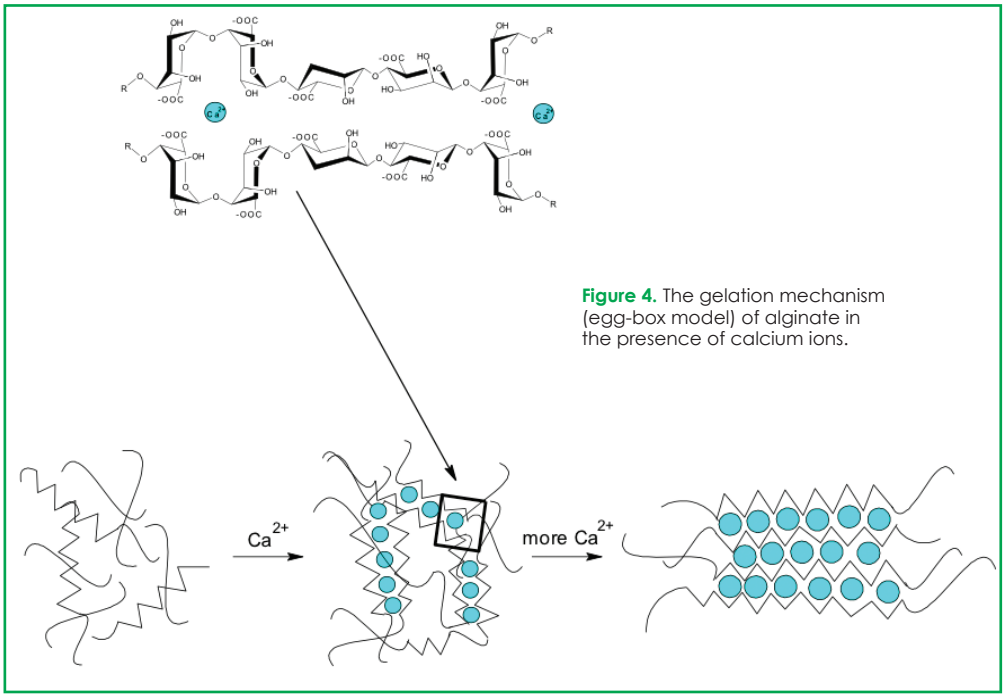

Figure 4. The gelation mechanism (egg-box model) of alginate in the presence of calcium ions.

containing traces of their biological precursors that do not contain the 3,6-anhydro-galactosyl residues and thus do not gel (7), modification of the seaweed hydrocolloids are performed prior extraction to increase gel strengths of the final products. The technique relies on addition of an alkali solution (usually KOH for carrageenans and NaOH for agar) that basically produces the 3,6-anhydro-galactosyl units accounting for the gelling properties of the hydrocolloids. The modified hydrocolloids are hereafter extracted with water at boiling temperatures. The extracted hydrocolloids are precipitated in alcohol and recovered by filtration and freeze-drying (22).

Alginate extraction can be done in different ways depending on application, but most commonly alginate is extracted as sodium alginate. Alginate is bound in the seaweed cell walls as insoluble calcium or magnesium alginate. Hence, the extraction technique relies on conversion of this insoluble alginate into a soluble compound. This solubilization is done by first converting the alginate into alginic acid and afterwards letting it react with sodium carbonate to form the soluble sodium alginate. Sodium alginate is precipitated in alcohol and recovered by filtration and freeze-drying (23).

SEAWEED CULTIVATION FOR HYDROCOLLOID PRODUCTION

Seaweed hydrocolloids are currently obtained from both cultivated seaweed species and via collection of wild types of seaweeds. The Philippines and Indonesia are the main cultivators of *K. alvarezii* and *E. denticulatum* for carrageenan extraction followed by Tanzania (Zanzibar) (24). However, wild cold water seaweed species such as *Gigartina* spp. and *Chondrus* spp. are harvested in Chile, Mexico, and Canada for extraction of carrageenans that cannot be obtained from cultivated, warm water seaweed sources (24). The only type of seaweed currently cultivated for agar extraction is *Graciliaria* spp., and the cultivation of *Graciliaria* for commercial agar production takes place mainly in Indonesia, China, and Chile. *Gelidium* spp., the other type of red seaweed used for agar extraction, is harvested mainly in Spain, Portugal, and Morocco as wild-types. *Laminaria* and *Lessonia* are the main brown seaweed species used for alginate extraction (24). *Laminaria* spp. are cultivated in China, Korea, and Japan, while the rest of the seaweeds used for alginate extraction are naturally harvested species from Norway, France, Chile, and Peru (13, 24). Seaweeds can be cultivated in ponds or tanks or in open-water on ropes, either tied to wooden pegs fixed in the sand near shore or tied to floating rafts further out in the sea. Cultivation methods are depending on the type seaweed species; red seaweeds are usually grown in intertidal zones whereas most brown seaweeds are grown in deeper waters (25, 26).

FUTURE OUTLOOK

The seaweed hydrocolloid market grows 2-3% per year and the demand has now surpassed the supply (13). Thus, there is an increased request for seaweeds holding functional hydrocolloids. There is a number of seaweeds that could be selected and even domesticated and developed as raw materials for hydrocolloid production; it is also an option to collect seaweeds locally as large quantities of unused wild seaweeds are found around the globe. A variation of different seaweeds is required as all the seaweed hydrocolloids have unique properties and thus have different uses. So far all attempts on commercial cultivation of *Gelidium* spp. for agar extraction have failed and the supply is currently accomplished by natural harvest which may induce depletion (27). Another issue when relying on collection of wild seaweeds for large scale hydrocolloid manufacture is the natural variation of the hydrocolloid contents in seaweeds of different age and size, accessibility, and costs associated with harvesting (13, 24). One way to overcome these issues could be to introduce new species for cultivation. One has to be cautious when adopting non-indigenous species as it may lead to deleterious effects on natural habitats or introduce invasive species. For cultivation to be financially viable, total hydrocolloid production levels have to be high (>1000 tons dry weight/year), and this has to be taken into account when considering cultivation of seaweeds (14). If a cost advantage over natural harvest is unreachable, one could consider promoting the use of indigenous species. In order to avoid depletion, as seen for *Gelidium*, it is important to consider long-term perspectives such as marine biodiversity and their ecological role as habitat and substrate for fish and other animals (29).

Canadian, and Latin American seaweed industries rely on the sustainable harvesting of natural resources. As several countries wish to increase their activity, the harvest should be managed according to integrated and participatory governance regimes to ensure production within a long-term perspective. Development of regulations and directives enabling the sustainable exploitation of natural resources must therefore be brought to the national and international political agenda in order to ensure environmental, social, and economic values in the coastal areas around the world.

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In Europe, Portugal requires an appraisal of seaweed management plans while Norway and Canada have developed and implemented coastal management plans including well-established and sustainable exploitation of their natural seaweed resources. Whereas, in Latin America, different scenarios of seaweed exploitation can be observed; each country is however in need of long-term and ecosystem-based management plans to ensure that exploitation is sustainable. These plans are required particularly in Peru and Brazil, while Chile has succeeded in establishing a sustainable seaweed-harvesting plan for most of the economically important seaweeds. Furthermore, in both Europe and Latin America, seaweed aquaculture is at its infancy and development will have to overcome numerous challenges at different levels (i.e., technology, biology, policy.

Alginate producers are also facing some difficulties regarding finding suitable seaweeds for alginate production due to a shift in market demands. In addition, brown seaweeds are expensive to cultivate, as they require deep waters, demanding vessels and manpower investment for monitoring and cultivation, and go through a reproductive cycle involving an alternation of generations (24).

Countries along the West African coast could be relevant in this regard, as many native seaweed species occur here and the climate is similar to current seaweed cultivation countries. In addition, seaweed manufacturing represents a unique opportunity to create local business opportunities, as already observed in Tanzania, where the introduction of the seaweed industry has created local business, increased employments and livelihood (30).

Studies have already been conducted on local Ghanaian red and brown seaweeds in order to access their potential to be used as future hydrocolloid sources. These studies have shown that several species could be considered for hydrocolloids production including the carrageenan containing red seaweed *Hypnea musciformis* and the brown seaweed *Padina* (22,31). Hence, *H. musciformis* prevalent in India, Brazil, and along the Atlantic coastline of Ghana, contains κ-carrageenan that has the same gelation properties as commercial κ-carrageenan from *K. alvarezii* (22), and *Padina spp*. harvested locally in Ghana harbors alginate that form very high strength gels, surpassing the gel-strength of commercial alginates from *Laminaria digitata* and *Macrocystis pyrifera* (31).

Since wild crops are somewhat unreliable and may vary in the composition and hydrocolloid levels, it could be relevant to introduce new cultivation technologies for the cultivation of the native hydrocolloid containing species. Seaweed cultivation is normally considered environmentally friendly, as seaweeds need no fertilizing and watering, but one important factor to consider when cultivating seaweeds, especially for human consumption, is their ability to take up nutrients from the surroundings. Due to the chemical structure of the hydrocolloids, the negative charges, they can strongly bind heavy metals from their surroundings, which has been exploited for e.g. cleaning of wastewaters. Lots of factors affect the heavy metal levels in seaweeds, including seaweed specie, age, water temperature, salinity, pH and nutrients concentrations (32). Relatively few studies have been conducted on the heavy-metal uptake of seaweeds, nevertheless it is an important aspect when considering new potential seaweeds as well as new cultivation sites. Hence, studies concerning heavy metal uptake by specific seaweeds at putative collection or cultivation sites, as well as detailed consideration of possible pollution of cultivation sites, are needed.

CONCLUSION

The seaweed hydrocolloid industry continues to grow and with an increased demand for hydrocolloid sources, i.e. seaweeds, there is an opportunity to create local business and increase livelihoods in third world countries located along the coast. West African countries such as Ghana have many native seaweed species that could be used as future hydrocolloid sources. As the use of wild seaweed species encounters issues such as reliability on continuous availability and potential depletion, it could be of interest to develop new cultivation technologies for these native species. As seaweeds have the ability to take up nutrients from their surroundings, potential new species and cultivation sites should be evaluated for chemical uptake and pollution in order to avoid high levels of heavy metals in the food hydrocolloids.

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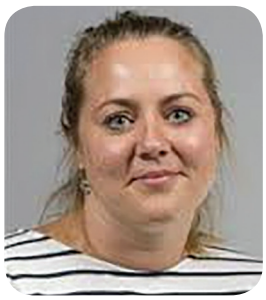

Nanna Rhein-Knudsen *is currently conducting her PhD study at the Technical University of Denmark on the hydrocolloids carrageenan, agar, and alginate extracted from red and brown seaweed sources. The aim of the PhD study is to characterize and utilize hydrocolloids from Ghanaian seaweeds and in particular try to optimize extraction methods by e.g. enzymes technology.*

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Food Reformulation

Maintaining nutrition in the elderly is more than just eating

There has been much work and research in the assessment child nutrition, and what is the optimal nutritive qualities that support a child's growth and development, which has resulted in a significant increase in ingredients that may be added to infant formula to mimic breastmilk. At the other end of the age continuum, research is gaining momentum on the nutritive needs of the elderly, and what factors lead to malabsorption in this age group. Malnutrition among the elderly has become a significant issue as the number of people aged 65 or older worldwide is poised to outnumber children, a first in recorded history [1]. Recent research has found that the inability to obtain adequate nutrition by the elderly has a variety of origins; not only that the elderly do not consume enough nutrient-rich foods. While a significant factor in nutrient malabsorption by the elderly is due to a decrease in food consumption, there are actually a number of underlying physiological issues that may be contributing to decreased food consumption by the elderly.

The flavorful taste of food is believed to enhance our quality of life, but Solemdal et al. [2] found that impaired taste among the elderly during an acute hospital stay was strongly associated with mortality, such that patients who scored highest on the ability to taste had an almost 70% better survival rate compared with patients with severely impaired taste. Non-drug induced decreased ability to taste among the elderly generally cannot be reversed, but intensification of the taste and odor of food may be able to offset some age-related perceptual losses [3]. This information indicates that food formulators producing food products marketed towards the elderly should increase the focus on strong, rich flavor profiles that may somewhat overcome this potential taste impairment. In addition, company-focused taste trials on elderly-focused food and nutrient supplement products should be conducted with a wide range of elderly subjects, to better reflect the potentially taste impaired elderly population.

The functional capacity of the gastrointestinal tract also changes as people age, and while some of the changes are considered typical of the age category, others may result in pain and an aversion to food consumption, and may also decrease the ability to absorb nutrients from food products. Studies have found that the ability of the esophagus to form or maintain the amplitude of normal peristaltic pressure waves while a person swallows decreases in the elderly, which results in gastroesophageal reflux and dysphagia (difficulty in swallowing). Starch-based food ingredients are often added to food or supplement products directed toward the elderly. Other gastrointestinal-related issues include a decrease in the gastric release of hydrochloric acid in the stomach and a diminished production/release of pancreatic enzymes (lipases and pepsin) and bicarbonate, which may result in decreased fat and fat-soluble vitamin absorption [4]. Other work has found that the gut microbiome may change as we age, increasing the potential for inflammation and decreasing the immune response, with an increase in *Clostridium difficile* infection, a common cause of acute diarrhea (and death) among nursing home residents [5].

The ability to absorb or produce certain nutrients also declines as we age. Aging decreases the formation of 1,25-dihydroxyvitamin D, the active form of vitamin D, by 50% as a result of age-related decreases in renal function [6]. Vitamin B12 has been found to be critical in the generation of new blood cells and in some aspects of neurological function. Vitamin B12 is a water-soluble vitamin absorbed through a highly complex process involving the stomach, pancreas and small intestine; loss or reduction in the function of these organs can result in impaired B12 absorption, leading to vitamin B12 deficiency. Severe B12 deficiency may result in pernicious anemia, neuropathy (tingling and numbness in the fingers/limbs) and myelopathy. Neurological effects include visual disturbances and memory loss, disorientation and finally dementia.

The decrease in nutrient absorption is not relegated to only those that are underweight, as people may be overweight or consume calories in excess, but still not absorb or metabolize nutrients critical to organ function and a healthy lifestyle. Recently, Park et al. [7] using a rodent model, found that as mammals age, their bodies produce increased amounts of DNA-dependent protein kinase (DNA-PK) in skeletal muscle, which suppresses mitochondrial function, energy metabolism and physical fitness. This increase is likely also occurring in humans, as these enzymatic pathways are similar in humans and rodents. The overall effect is a metabolic and fitness decline during aging, potentially contributing to weight gain and lowered metabolic rates, which increases the likelihood of other diseases such as insulin resistance and bone fractures. While an inhibitor of the DNA-PK enzyme is a long way off, this work shows that the nutritive demand of an aging population requires more nutrient-dense, low-calorie foods, compared to a younger population.

Development of food supplements containing readily absorbable, nutrient-dense vitamin/mineral formulations (e.g., nanoemulsions), probiotic formulations and other nutrient-rich supplements may be critical in decreasing

nutrient deficiencies in the elderly. While these novel formulations will require toxicological testing and safety assessment evaluations (certain vitamins and mineral consumed at high doses can be toxic), the increase in a vibrant, functional elderly population will outweigh initial investment costs. As the world's population ages, the role of the food industry will be significant in keeping the elderly a healthy and productive part of society.

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MINERVA: the technological project to boost research on the microbiota-gut-brain axis in chronic neurodegenerative disorders

KEYWORDS: Intestinal microflora, neurodegeneration, microbiota- gut-brain axis.

Abstract Chronic neurodegenerative disorders including dementias, as Alzheimer's disease (AD) and movement disorders, as Parkinson's disease (PD) are strongly increasing worldwide. Up to now, very little is known about their biochemical mechanism and no effective therapeutic strategies are available to face brain functionality deterioration. A very intriguing hypothesis actively under investigation is referred as "microbiota-gut-brain axis". It suggests that human gut microbial community might impact central nervous system functionality through a bidirectional interaction that potentially might play also a key role in neurodegenerative disorders. Here we present a novel technological project, named MINERVA supported by the European Research Council (ERC) Programme, that aims, by using an innovative bioengineering approach, at evaluating microbiota impact on brain functionality. MINERVA goal is to develop a cutting edge technological platform, based on organ on chip microfluidic device, to model the main players of the microbiota-gut-brain axis. The final aim of MINERVA is to investigate the role of microbiota on brain functionality in physiological but also in pathological neurodegenerative conditions in order to improve the knowledge in the field and open the way to potential novel therapeutic microbiota-based approach for neurodegenerative disorders.

MICROBIOTA-GUT-BRAIN AXIS: A FOCUSED OVERVIEW

Alzheimer's (AD) and Parkinson's (PD) disease are severe chronic neurodegenerative pathologies. ADfeatures memory loss, followed by behavioral changes and impairment in everyday life. PD is characterized by movement disturbances, but it is not unusual the presence of dementia (1). An extremely interesting hypothesis is that the intestinal microflora (collectively named "microbiota") is a key player in chronic neurodegeneration. A relation between gut microbiota and brain, referred to as "microbiota-gut-brain axis", was firstly hypothesized more than 100 years ago by the Russian embryologist Elie Metchnikoff: he surmised that a healthy microbiota could help counteracting aging and that some bacterial strains (what we call today "probiotics") found in sour milk and yogurt would increase longevity (2). Literature shows that Metchnikoff's findings have been recently re-evaluated, becoming a new, exciting hypothesis in neuroscience: up to now, the papers on a possible role of gut microbiota on brain or AD/PD increased impressively, going from 8 in 2008 to 842 in September 2017, with 258 articles published in 2016 only (Table 1; source: PubMed). Many proposals dealing with the microbiota have been granted in the last 6 years: in 2011 and in 2012, the Michael J. Fox Foundation (USA) funded two grants on gut microbiota effect on PD, while "MYNEWGUT" was financed in Europe and it deals with diet, microbiota and its impact on brain development. The Human Microbiome Project-HMP (USA) was designed to sample, determine and quantify all human-associated microbiota; MetaHIT in Europe, is focused on intestinal microbiota; Eldermet in Ireland, is a national project associated with the large European project "NU-AGE" (the latter centered on the elderly) aimed at assessing the association between gut microbiota, food and health in the elderly. Very recently, the H2020 EU Programme granted the "AD-GUT" project, where for the firsttime microbiota composition will be manipulated in AD patients to evaluate how this affect the diagnosis and progression of the disease.

Year	Microbiota gut brain (MGB) *(number of publications)*	MGB and Alzheimer's disease *(number of publications)*	MGB and Parkinson's disease *(number of publications)*
2008	4	0	0
2009	8	0	0
2010	16	0	0
2011	30	0	0
2012	31	0	0
2013	67	2	0
2014	142	3	0
2015	174	7	7
2016	258	11	11
2017 (September)	229	21	23

Table 1. Number of published articles on the microbiota-gut-brain axis in the last 10 years, with a focus on Alzheimer's disease and Parkinson's disease. (source: PubMed; keywords: "microbiota gut brain"; "microbiota gut brain Alzheimer's disease"; "microbiota gut brain Parkinson's disease".

Note: the sum of published article/year exceeds the single search from 2008 to 2017 (842, 37 and 38, respectively) as some papers in the single year search are counted twice (year of e-pub and printed version).

NEURODEGENERATIVE DISORDERS AND MICROBIOTA IN LITERATURE

We have already several studies addressing the role of gut microbiota in (AD) or (PD) disease. For example, Bhattacharjee et al that described a number of evidences supporting microbiota-AD relationship (3). In addition, Wang et al reported a role of the intestinal microbiota in the protective activities of polyphenols in AD (4), and a similar conclusion was drawn in a mouse model of neurodegeneration (SAMP8 mice) (5). A direct action of microbiota on mechanisms involved in AD was reported by Minter et al, that suggested in mice a regulation by the gut microbiota community of host innate immunity mechanisms, with an impact on inflammation and amyloidosis (6). Many other researchers reported a link between gut microbiota and AD in animal models (7-10). A very important finding came from a seminal work in human patients, where it was reported an increase of a pro-inflammatory microbiota in patients with cognitive impairment and brain amyloidosis (11). As for PD, researchers have already found a different microbiota composition in PD patients in comparison to controls, (12-14), while PD animal models point to a role of microbiota in clinical signs, as reported by Sampson et al, whose findings suggested that gut bacteria regulate movement disorders (15).

Disease	Animal models	Clinical studies	*In vitro* cell based models
Alzheimer's Disease	-Transgenic / Germ free mice mice (6-8) Invertebrate models (Drosophila, C. elegans) (9)	-AD patients compared to healthy controls (11)	*In vitro* model of amylod formation (4)
Parkinson's Disease	- Transgenic / Germ free mice (15)	-PD patients compared to healthy controls (12,13)	

Table 2. Examples of current models to address the role of gut microbiota in Alzheimer's and Parkinson's neurodegenerative disease.

CURRENT AND INNOVATIVE MODELS TO STUDY THE IMPACT OF MICROBIOTA ON BRAIN

Currently the main experimental findings on microbiota-gut-brain axis impact on brain functionality, and in particular on AD/PD, rely mostly on *in vivo* assessments in rodent models, that allow to investigate the whole-body response to selected stimuli. Some of them have been used in particular to asses microbiota role in neurodegenerative disorders such as AD and PD (Table 2). However, they have several limitations: (a) rodent's microbiota is very different from the human one, (b) even if we transplant human microbiota in rodents, residual murine or environmental contamination is a major issue, and (c) they are expensive, lab-intensive and do not allow a strict control on microbiota composition once inoculated. Many papers are also based on *in vitro* studies, but they are performed with standard techniques that rely on 2D static immortalized cell models and their treatment with bacterial-derived molecules, a model that is very far from the real in vivo situation. In this setting, the availability of valuable innovative technological tools to consolidate the potential mechanisms by which the microbiota impacts the brain functionality might greatly help in overcoming the current limitations of *in vitro* and *in vivo* tools (16). Actually, some interesting dynamic digestion model that involves microbiota component and reliably reproduce some key features of the human digestive tract are available, in particular: the Simulator of Human Intestinal Microbial Ecosystem (SHIME™ and M-SHIME™) (17); the TNO Gastro-Intestinal Model (TIM) (18); HUMIX (19). However, the study of the impact of gut microbiota modifications on brain functionality is far from being their aim.

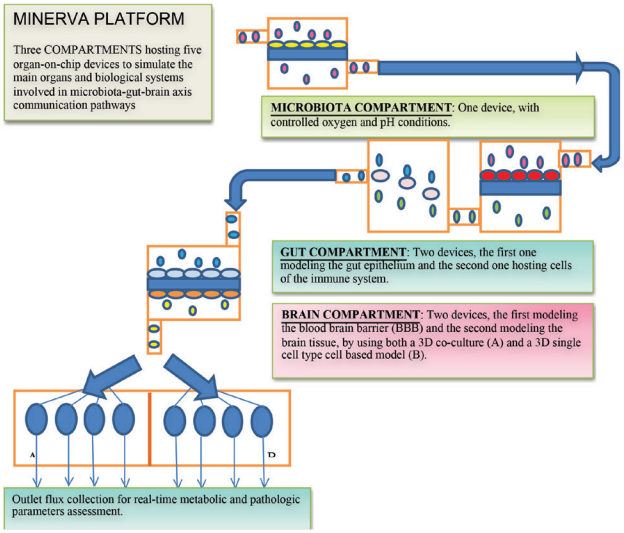

Figure 1. MINERVA platform: it is based on three main compartments, representing the microbiota, the gut, and the brain that hosts five optically accessible, organ-on-chip microfluidic devices. Each device hosts advanced in vitro cell models to model the main players of the microbiota-gut-brain axis: the microbiota; the gut epithelium; the immune system; the blood brain barrier; the brain.

MINERVA: AN ERC TECHNOLOGICAL PROJECT TO EVALUATE MICROBIOTA IMPACT ON BRAIN FUNCTIONALITY

Recently, an ERC Consolidator project named "Microbiota-Gut-Brain engineered axis to evaluate microbiota impact on brain functionality" (acronym: MINERVA, ERC-CoG-2016 - Proposal 724734), was funded and aims at designing the first complete "microbiota-gut-brain" axis physical model by using a bioengineering approach. This innovative platform relies on three compartments, based on state-of-the-art miniaturized, optically accessible microfluidic devices, hydraulically connected to reproduce *in vitro* the microbiota-gut-brain axis connection (Figure 1). In the MINERVA platform, human microbiota will be cultured in the "Microbiota-compartment" and the secreted molecules (the so called "secretome") transported to the "Gut-compartment" where gut epithelial cells and cells from the immune system will metabolize them as occurs *in vivo*. The resulting modified secretome will reach the "Brain-compartment", built up by a complete blood-brain barrier *in vitro* model followed by two exhaustive 3D human brain cell models, featuring the three main populations of brain cells: neurons, astrocytes and microglia. In the first model, the cells will be co-cultured, to recapitulate brain cell-to-cell contacts; in the second model,

each cell type will be cultured individually to let explore the microbiota's effect on each cell type. MINERVA platform will be functionally and biologically validated using an approach at increasing complexity, to finally assess neural cell response once exposed to human complete microbiota from (a) healthy donors, and (b) Alzheimer's disease (AD) patients. MINERVA has the potential to fill the gap existing in current tools in the field of microbiota-gut-brain axis at the boundaries between *in vitro* and *in vivo* models, to deep our knowledge in the field and suggest innovative strategies to be tested after the approval of clinical studies enrolling AD/PD cases that ultimately will demonstrated the impact of microbiota control in neurodegeneration.

CONCLUSIONS

The fundamental concept of an interaction of gut microbiota with the nervous system has been revisiting in recent years: the main novelty is the search for a causal link between microbiota composition or metabolism and the molecular determinants of pathologies as Alzheimer's or Parkinson's disease, mostly considered up to now as based on brain autonomous mechanisms. We have very limited knowledge in this field but studies and models are under development. This hopefully will boost the perspective of a clinical translation, as the microbiota-brain relation would represent a breakthrough also from the therapeutic point of view: human microbiota composition is dynamic and tunable by diet and probiotics, a very common tool in medicine, for instance in association with antibiotics therapy.
It is out of doubt that the challenge is complex and only a multidisciplinary approach based on fruitful discussion among neuroscience, immunology, gastroenterology and bioengineering will be successful and MINERVA might represent a first challenge on this way.

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Consumer perspective

What drives food choice?

To understand why we eat what we eat we need to understand what makes us choose and buy different food and drink products. What are the drivers behind our choices?

EUFIC (the European Food Information Council) has looked at consumers from five different countries (UK, France, Italy, Poland and Turkey) and their choice behaviour for a number of different product categories.

The two main themes that have emerged are "indulgence" and "health". Consumers are looking for an enjoyable consumption experience but they also link that consumption to a number of health benefits. The way these links are established is interesting, though. Contrary to what one might expect, it isn't always the actual healthfulness (i.e., nutritional value, health benefits, portion size etc.) that makes consumers associate specific products with a health benefit. Rather, we find that it's the way food makes us feel when we consume it. Take a look at the following line of reasoning: "when I enjoy eating a tasty food, it puts me in a good mood and helps me relax after a long, stressful day – and this moment of relaxation is *good for my health*". Long working hours, hectic schedules and pressure in daily activities can lead to people feeling stressed out, exhausted and overwhelmed.

We know that food and drink products serve more purposes than just to feed, satisfy an appetite or satiate hunger. They make people happy, they reward, they help build connections in social groups – and now it's becoming clear that they also unlock what consumers perceive are health benefits. However, this link between indulgence and health can be two-fold: while in some cases we see that indulgence can lighten the mood and be seen as a positive influence on one's personal health, in other cases it is a good taste that unlocks health benefits of a product because the tastiness ensures consumption.

But there is more. Food products can take on different roles in different countries. Take biscuits for example: In the UK, biscuits are seen as a treat that leads to enjoyable consumption. For Italian consumers, biscuits are also about indulgence – but there is a functional role here, too. Biscuits are often consumed for breakfast because they provide energy and help people in their daily lives. French consumers agree that it's the enjoyable consumption that drives most of their product choice – but they explain part of this indulgence to be associated with their childhood memories. For Polish consumers, enjoying the consumption of biscuits provides them with comfort and relaxation which improves their productivity and allows them to earn more money.

Country differences for one and the same food product point to cultural differences in the role food plays in our lives. Notably, there is a foods-as-nutrients approach visible in the UK data with consumers being largely driven by health considerations when choosing their foods. In comparison, Italian consumers are more balanced between health and indulgence motives, plus they display an interest in brands, specifically to support local or national production. French consumers focus mostly on indulgence and display a more holistic approach out of all five countries: they are largely driven by a desire for well-being. For Polish consumers, we find that health is the strongest driver of food purchases. But once again, different to what one might think. Across different product categories, foods take on the role of feeding the body and providing energy so people can go to work, be more productive and earn more money. Here, food is 'fuel'!

This study also looked at Turkish consumers but these findings are much more complex. Very few clear paths could be identified and while both indulgence and health were part of the conversation, no clear themes of food choice emerged. The complexity of the data could point to a high involvement with food within Oriental culture and tradition. This could refer to the importance of hospitality, wanting to take care of others and one's family. But there are also strong indicators that Turkish consumers strive for acceptance, recognition and self-esteem/-confidence when choosing their food products. Such drivers would be very different from the typical drivers of food choice we see in other Western countries and present interesting new paths for research.

The one thing all participants across the five countries could agree on was what they ultimately aimed for: happiness. For most foods in most countries, happiness was considered the end-state – the desire all consumers wanted to fulfil. And if food can contribute to this, then we're a step closer to understanding consumption.

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Please send your reaction/comments/topics you would like to analyze to Dr Gayle De Maria at gayle@teknoscienze.com placing in the subject line "Consumer perspective column".

Industry perspective

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Technologies and new business models to increase sustainability in agro-food value chain

Promote quality and reduce environmental footprint in durum wheat cultivation processes

KEYWORDS: Multi-stakeholders, agricultural technology, crop rotation, carbon footprint, cost saving.

Abstract Taking into account the growing demand for food and the new social and climate challenges, agro-food systems must improve their efficiency by catching solutions in a 'from field to fork' perspective. A common agenda amongst agro-food players such as farmers, industries, retailers and consumers, should identify suitable sustainable initiatives to increase food safety and security and limit use of natural resources and their exploitation.
In this context, the Barilla Sustainable Farming Project (BSF) was developed in Italy since 2012 in cooperation with researchers, farmers and their advisors, along with other stakeholders. The main outcomes of the BSF are the "Handbook for the Sustainable Cultivation of Quality Durum Wheat", a new "sustainability tool" in a decision support system (DSS) called granoduro.net® and the design and use of a set of new durum wheat cultivation contracts. This paper shows by comparative analysis, that the use of an integrated system approach, as facilitated by the BSF project, can provide better performances in terms of cost saving for the farmers, standardization of high product quality for the industry and reduction of the environmental impact for the society, as a whole.

INTRODUCTION

Since the '70s, agricultural systems have focused on maximizing productivity by adopting new technologies and modernizing production techniques, such as using high-yield plant varieties, monoculture, mechanization and agrochemicals (1, 2). This intensive model has accelerated natural resources exploitation, compromising the biosphere regeneration capacity and, indirectly, affecting future agricultural productivity (3).
Looking at the increasing concerns in the society regarding the environment, food safety and food security, these issues are increasingly acknowledged by international organizations, politicians and the civil society, now called to stimulate a change in food production and consumption patterns (4). Reaching sustainable agricultural systems is recognized as one of the most important drivers to guarantee global food availability in the future (5, 6).
In response to these global challenges, there is increased awareness about the urgency to enhance resilience and sustainability of the agricultural systems, as well as to mitigate the environmental impact of food production and distribution at a global scale (7). According to several definitions, the agro-food sector is complex and its output is strictly connected to specific socio-ecological elements and local agricultural systems (1, 8).
In the last few years some food companies started promoting dialogue amongst farmers, technical advisors, researchers and consumers to better understand how they could move in the sustainable pathway (9-11). Indeed, several companies started putting greater attention to consumer behaviour, in some cases, by promoting education schemes and social messages useful to support environmental impact reduction in the downstream value chain (12).
In this perspective, this paper suggests a new participatory innovation strategy within dry pasta value chain developed by a global leader company in the pasta market, one of the most historical Italian food companies, Barilla G & R Fratelli. Looking at a sustainable farming project promoted by Barilla, some results are herein discussed, to indicate a possible way to embed sustainability into the agro-food chain.

BARILLA SUSTAINABLE FARMING INITIATIVE

The sustainability pathway

In the last 20 years, Barilla strengthened its Corporate Social Responsibility and Green Labelling activities in response to consumers' increasing environmental and social concerns. In the same period, many actions were undertaken by global food companies towards agro-food sustainability (13, 14). Most of these initiatives were preliminary actions aimed at introducing certification

schemes and encouraging the implementation of sustainable practices in supply management, to enhance participation of supply chain actors and raw material providers in very different contexts in the world (15).
Since 2000, Barilla has conducted a Life Cycle Assessment - LCA analysis to better understand how its portfolio of products could be redesigned to reduce their environmental impact. In collaboration with Life Cycle Engineering, an Italian engineering start-up, Barilla started conducting LCA on several of its products. In 2008, Barilla obtained Environmental Product Declaration for pasta and some other products and confirmed that the greatest environmental impacts belonged to raw material production (crop cultivation). Based on such evidence, in 2009 Barilla decided to develop a specific initiative to reduce the impact derived from durum wheat production, its most relevant raw material in terms of volumes purchased (16).

From observation to action

As cultivation sustainability is strictly connected to interactions amongst soil, plants and agro-ecological conditions, a specific study was conducted to determine whether different crop rotations could help in increasing the sustainability of the whole system (17, 18). A research project was conducted to analyse and compare different cropping systems and quality performances in several durum wheat cultivation areas, located in the North, Centre and South Italy. Since project beginning, some collaborations were developed, such as with Horta, an academic spin-off specialized on agronomics and innovations in cropping systems management. This cooperation made it possible to define and test, through pilot experiments, the outcome derived from different crop rotations, managed and monitored by an innovative technical decision support system - DSS developed by Horta, called granoduro.net®. (19). The test was initially conducted in 13 farms. To verify the effectiveness and feasibility of the suggested practices, farmers agreed to cultivate part of their land following some simple agronomic rules and granoduro.net® indications; another portion of land was managed by farmers in their usual way (20). The first results, obtained in 2011/2012, were encouraging. The indicators showed that, compared to common cultivation practices, thanks to the combined use of DSS and crop rotation, it was possible to reduce up to 30% greenhouse gas emissions as well as to increase production yield up to 20% and to obtain a 30% reduction in cultivation production cost.

BSF at a glance

The positive results obtained through pilot experiments confirmed that the adoption of proper rotations combined with the use of DSS technical indications reduced the environmental impact and cultivation production cost and improved grain quality, compared to the most common farming techniques generally adopted in each production area.
Following upon these results, Barilla developed a series of recommendations for the sustainable cultivation of durum wheat and summarized them in the 'Handbook for Sustainable Cultivation of Quality Durum Wheat'.
In 2012-2013, Barilla Sustainable Farming (BSF) project started involving 25 farms from the same durum wheat cultivation areas used in the pilot. The project team decided to enforce the evidence obtained and to use the data to define a new supply chain model in line with the "Good for you, good for the planet" company business strategy.
A large-scale project promoting sustainable agriculture practices originated from Barilla's teamwork with research institutions, cereal suppliers, farmers' cooperatives and organizations, LCE and Horta.
BSF is promoting:

- R&D projects finalized at designing a specific tool to collect and explore primary field data about fertilizer and plant protection use, to improve farmers awareness on input efficiency and to improve environmental impact assessment;
- suppliers training days, focused on DSS use, also useful to collect feedback from experts about DSS suggested choices' feasibility;
- "Campi Aperti" (Open Fields), farmers dissemination initiatives to improve their awareness about cultivation efficiency, DSS utilization, also suitable to understand farmers perceptions and willingness to adopt BSF innovations;
- definition of a cultivation contract called "High quality durum wheat contract" along with the design and test of an innovative multi-years cultivation contract, thanks to the definition of clear partnerships with other industrial actors, in specific with tomato, cereals/ vegetable oils and sugar beet processors in Central and Northern Italy.

CULTIVATING SUSTAINABILITY BY TECHNOLOGY AND TRUST CHAIN

The handbook for Sustainable Cultivation of Quality Durum Wheat

The handbook consists into a list of 10 guidelines for durum wheat cultivation, aimed at allowing farmers increasing the crop yield, food safety, fertilizer use efficiency, income, quality and decreasing the crop environmental impact.
In particular, the rules encounter: adopting intercropping/ crop rotation and the most appropriate crop management/tilling techniques according to specific soil conditions, using the most suitable variety, using certified and treated seeds in the correct amount, sowing at the right moment, controlling weeds promptly, dosing nitrogen in relation to plant needs and soil structure, protecting plants from disease according to risk conditions defined by specific meteorological and environmental data.
The Handbook novelty is to decline crop management indications according to specific local soil and climate conditions. Also, it suggests concrete solutions to adapt crop management to Common Agricultural Policy indications.

Information Technology, DSS and environmental accounting

Developed by Horta s.r.l, a spin-off company of the Catholic University of Piacenza, granoduro.net® consists into a web-assistance service which integrates information on weather, soil and crop variety characteristics. DSS firstly collects, organizes and integrates all types of information

required for monitoring crop growth and development. Secondly, the system analyses information gathered to make objective deductions and recommend users the most appropriate actions to undertake. The platform allows farmers optimising their operative decisions about seeding, fertilisation, weed control, pests & disease management. Information is punctual and provided for crop unit, defined as a portion of soil with same characteristics in terms of variety, preceding crop, soil texture and cultivation technique (19).
The tool used to collect information from farmers is the 'Crop Operations Register', a standardised framework where crop data are included day by day, allowing great organisation and easy availability for all users, at any stage of crop development.

High-Quality Durum Wheat contract

In the agro-food industry, contract farming is growing as a way to mitigate demand uncertainty for farmers and supply uncertainty for industrial buyers. In Barilla, durum wheat supply contracts evolved across time and they are currently recognized by the Italian Ministry of Agriculture and several Italian Regions as good opportunities to mitigate price volatility in durum wheat production areas (21).
To ensure the high quality durum wheat needed in pasta production, Barilla passed from signing with suppliers variable-price contracts in 2006, to contracts with additional premiums in 2009, to fixed-price contracts based on farmer cost of production in 2010, to the introduction of a price mechanism based on common wheat market prices in 2012 and, since 2013, to sustainability contracts, guaranteeing additional premiums for protein content and for the adoption of the handbook, disciplinary, exclusive varieties and granoduro.net indications (22).

RESULTS AND NEW CHALLENGES

Sustainable intensification and efficiency

To analyse the real effects of the new tools on durum wheat cultivation sustainability, Barilla suppliers (grain elevators and farmers' cooperatives) involved in BSF must collect soil and field management data from farmers and input them into granoduro.net®. This data mining allowed, across time, monitoring specific indicators used to evaluate BSF performance across the years in each area. These indicators quantify modifications in cropping systems in response to different management indications. To summarise and communicate annual results to different stakeholders, crop cultivation data are collected by DSS crop operations register and elaborated to calculate environmental indicators (carbon footprint, water footprint and ecological footprint), agronomic indicators (yield and Nitrogen Use Efficiency), food safety indicators (DON index) and economic indicators (direct costs, gross margin and net income) (23).
The pilot experiment positive trend was confirmed across the years, showing that BSF integrated systems can actually improve for all indicators, such as: reduction in farmer direct costs and in carbon footprint (Figure 1, 2), along with relevant increase in yield, NUE and gross income. Since 2012, this system always succeeded in reducing the environmental impact of durum wheat cultivation, as well as in increasing the gross margin and the revenue for the farmers, while strengthening the resilience of the agro-ecosystem, especially during critical seasons.

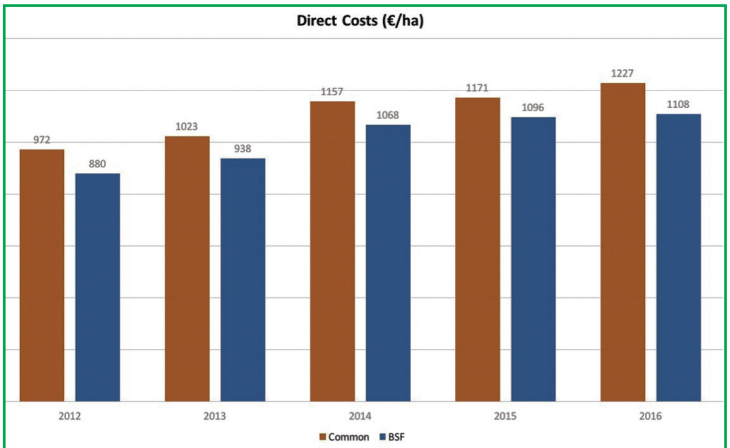

Figure 1. Comparison between on average durum wheat production costs calculated on BSF farms that using both traditional and BSF systems.

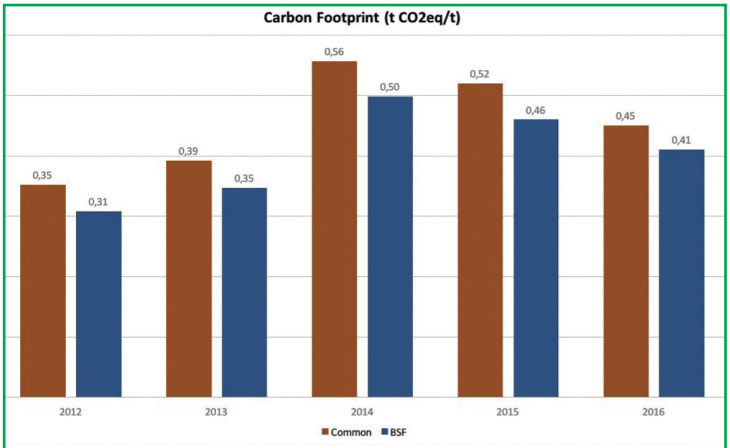

Figure 2. Comparison between on average durum wheat Carbon Footprint calculated on farms that using both traditional and BSF systems.

BSF trends and diffusion

These positive results find confirmation in the number of BSF participants and durum wheat quantity obtained through High-Quality contracts, both increasing across time. Starting from 25 farms in 2012, in 2016 BSF involved more than 30 suppliers, accounting for about 2,000 farms and more than 76,000 durum wheat hectares. Barilla increased its purchase of high quality and sustainable durum wheat from most vocated production areas in Italy, passing from 10,000 ton to more than 190,000 ton. The objective is to achieve 400,000 t of durum wheat produced using these tools by 2020 (24).
The above results confirm that the interaction amongst industry, farmer organizations, suppliers and extension services represent a multiplier leverage element. Where this collaboration is anchored to long term trust, the number of adopters show a rapid increase and, in some cases, start catching the interests and support of the local government and political institutions.
In recognition of its efforts, Barilla won the European CSR Award in 2013 and started applying the BSF experience in other countries where it currently operates (Greece, Turkey, Canada and USA). New collaborations and teambuilding

activities were conducted in each country, involving local research institutions and farmer associations.

DISCUSSION AND CONCLUSION

BSF project clearly showed that sustainability should always be addressed considering all aspects at the same time: environmental protection, supply security, food safety and adequate revenue for all actors involved in the value chain.
BSF main results, consisting into suggesting farmers to adopt the most suitable crop rotations along with sustainable cultivation practices, allowed Barilla also undertaking an innovative approach to contracting, leading to the generation of new 'horizontal agreements' with industrial partners from different supply chains, thus incentivizing farmers to adopt crop rotations, linked to the guarantee of crops commercialization (25). To enhance these relationships, in 2016, Barilla introduced three-year cultivation contracts, as opposed to annual ones, to reward virtuous Italian farmers, fostering and promoting local durum wheat production and improving sustainability.
Thanks to this brand new concept, for the first time, while still putting the farmer at the centre, a new logic of 'cooperating to compete' is adopted, by offering concrete business opportunities in a 'win-win' situation for all parties to reduce the agricultural impact and contribute spreading a new thinking regarding sustainability.

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Palm oil: health risks and benefits

KEYWORDS: Palm oil, tocotrienols, saturated fatty acids.

Abstract Recently, there has been growing discussion on the use of palm oil in the preparation of food products. In this review, we first examine the criticism of palm oil, in particular the supposed contribution to increase cardiovascular disease risk factors because of the high content in saturated fatty acid and the content of potential toxic compound formed during the refining processes, such as glycidols and chloropropanediols. The results of the main publications on these topics demonstrate that the massive attack against palm oil is largely unjustified if palm oil is assumed within the limits of a balanced diet. Finally, we discuss the possible benefits on health of the minor components of palm oil, such as tocopherols, tocotrienols and carotenoids.

INTRODUCTION

Recently, there has been growing discussion on the use of palm oil in the preparation of food products. The purpose of this review is to examine the criticism of palm oil and to discuss its possible benefits on health.

Palm oil accounts for one-third of global plant oil production because of its very high versatility as food ingredient free of *trans* fatty acids (*t*-FA) and its low cost. It is obtained mainly from *Elaeis guineesis*, a palm native of West and Southwest Africa; however, in recent years its cultivation has intensely spread in Southeast Asia, especially in Malaysia and Indonesia. The enhancement of palm tree cultivation has prompted a number of concerns because of its contribution to deforestation. Although the impact of palm oil production on deforestation is beyond the scope of this review, we wish to point out that the yield of oil per hectare of palm crops is 3-8-fold higher than that of the alternative cultivations for oil production, such as sunflower, soybean, rapeseed and corn (1).

Crude palm oil (CPO) derives from the fibrous mesocarp of the fruit, is reddish in colour because of a high content in natural carotenoids and is the richest natural source of tocotrienols, but is unpalatable. CPO processing through three refining stages, namely degumming, bleaching and deodorising, give rise to refined, bleached and deodorized (RBD) palm oil. The normal refining process determines the loss of almost all carotenoids, but preserve the majority of tocotrienols (2). Before or after refining, palm oil can be fractionated into liquid (olein, 70–80%) and solid (stearin, 20–30%) components. Most of European producers first refine CPO and then fractionate it. RBD palm olein is used in frying, cooking, shortenings and margarines. RBD palm stearin is used mainly in food applications that require higher solid fats content such as shortenings and margarines (3). Fatty acid composition of red palm oil (RPO), palm olein (PO), palm stearin (PS), and sunflower, soybean, corn and olive oils are resumed in table 1, the values of fatty acids have been obtained from different bibliographic sources (4-6). PO, the fraction mainly used in food industries, contains approx. 45 % of saturated fatty acids (SFAs), 44 % of monounsaturated fatty acids (MUFA) and 11% of polyunsaturated fatty acids (PUFA); moreover, MUFAs consist mainly of oleic acid (18:1, 44% of total fatty acids) and SFAs of palmitic acid (PAc, 16:0, 40% of total fatty acids). PS is the fraction of palm oil richest in SFAs (55%) and PAc (49% of total fatty acids). Among vegetable oils reported in table 1, palm oil and its fractions are the richest in SFAs and PAc, whilst olive oil has the higher concentration of oleic acid, and, sunflower, soybean and corn oils have relatively higher contents of linoleic acid (18:2).

	Red Palm Oil	Palm Olein	Palm Stearin	Sunflower	Soybean	Corn	Olive
Fatty acids (%)							
Total SFA (%)	47.1	45.06	55.21	14.1	20.1	19.3	21.9
C14:0	1	1.02	1.14	0.1	0.1	nd	nd
C16:0	41.21	39.66	49.42	9.1	14.6	16.1	15.9
C18:0	4.34	3.81	4.15	4.6	4.9	2.6	5.4
C20:0	0.4	0.34	0.3	0.3	0.5	0.6	0.6
Total MUFA (%)	38.92	44.19	36.3	26.7	21	28.7	69.9
C16:1	0.37	0.18	0.16	0.1	0.1	0.1	1.2
C18:1	38.45	44.01	36.14	26.4	20.6	28.3	68.4
C20:1	0.1	nd	nd	0.2	0.3	0.3	0.3
Total PUFA (%)	12.58	10.93	8.25	57.9	58.3	51.8	8.2
C18:2	9.28	10.73	7.95	57.8	50.5	50.6	7.2
C18:3	3.3	0.2	0.3	0.1	7.8	1.2	1

Table 1. Fatty acid composition of red palm oil, palm olein, palm stearin, and sunflower, soybean, corn and olive oils.
RPO fatty acids (4), PO and PS fatty acids (5), sunflower, soybean, corn and olive oils (6).

POTENTIAL HEALTH RISKS

The main potential health risks ascribed to massive use of palm by food industries regard its high content in SFAs that can increase cardiovascular disease (CVD) risk and the presence of potential toxic contaminants that can accumulate in the body.

Current Italian nutritional recommendations for fat suggest that the total intake should range between 20 to 35% of the total energy in adult and from 35 to 40% in infants and young children, while SFAs should not overcome 10% of total energy and *t*-FAs as little as possible (7). However, the optimal intake of total fats and individual fatty acids for CVD prevention is still matter of debate in the scientific community. The classic diet-heart hypothesis made in the '70s that a high SFAs intake increases the risk of CVD by raising serum cholesterol has not always been confirmed by more recent findings. A meta-analysis carried out in 2011 by Siri-Tarino et al. (8) has not provided evidence that a high intake of SFAs is associated with an increased risk of coronary heart disease. More recently, an epidemiological study on the association between dietary habits and mortality indicated that high intake of all type of fat was associated with a reduced risk of mortality and was unrelated with CVD or CVD mortality, and, surprisingly, that SFAs had an inverse association with stroke (9). *t*-FAs formed from unsaturated fatty acids during food processing at high temperatures or in the production of partially hydrogenated fat (10) have been associated more strongly with CVD risk than SFAs (11). Substitution of partially hydrogenated fat with PS has considerably lowered *t-FAs* contents in packaged feed. A meta-analysis of dietary intervention trials on palm oil and blood markers of CVD found that palm oil determines both favourable and unfavourable changes when was used as an alternative for the primary dietary fats, whereas induces only favourable changes when was employed as a substitute of *t-FA* sources (12). Considering the above, it seems reasonable to presume that palm oil should not appreciably influence CVD risk factors if assumed within the limits of a balanced diet; nonetheless, additional studies are needed to better clarify this issue.
Finally, as concerns the potential toxic contaminants, palm oils contain fatty acid esters of glycidiol (G-FE) and fatty acid esters of monochloropropanendiol (MCPDE), in particular 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,2-diol (2-MCPD), formed during refining processes (13). However, these compounds are formed during the refining processes of all seed oils, and for the European population the main source of MCPDE are baked goods, where they are synthesized also from the glycerol produced by the action of yeast enzymes (14). Glycidol is categorized as probably carcinogenic to humans, whereas 3-MCPD and its fatty acid esters cause progressive renal and sperm toxicity in long-term animal studies; unfortunately, there are no data on long-term studies for 2-MCPD or 3-MCPD fatty acid esters (14). Currently in Europe the assumption of these harmful compounds is not regulated, however a draft regulation on G-FE limits in all oils, infant formulae and infant food has just been proposed and should come into force in 2018. The maximum levels proposed for G-FE expressed as glycidol are the following: 1000 µg/kg for vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food; 500 µg/kg for vegetable oils and fats destined for the production of baby food and processed cereal-based food; 75 µg/kg until 30.06.2019 and 50 as from 1.07.2019 for infant formula, follow-on formula and foods for special medical purposes intended for infants and young children (powder); and 10.0 µg/kg until 30.06.2019 and 6.0 µg/kg as from 1.07.2019 for infant formula, follow-on formula and foods for special medical purposes intended for infants and young children (liquid) (15). The content of these compounds in refined oils strongly depends on the refining processes (16); thus, it would be desirable, that the improvement of food industrial processes further reduces the concentrations of these dangerous compounds not only in palm oils, but also in the other several different foodstuffs containing them.

HEALTH BENEFITS

	Red Palm Oil	Palm Olein	Palm Stearin
Tocols (mg/kg)			
α-Tocopherol	**188.2**	**179**	**50**
γ-Tocopherol	**nd**	**17.6**	**nd**
α-Tocotrienol	**198.1**	**219.9**	**47.4**
β-Tocotrienol	**10**	**8.1**	**9**
γ-Tocotrienol	**198.8**	**332.7**	**134.9**
δ-Tocotrienol	**98.4**	**67**	**31.4**
Carotenoids (ppm)	**630-700**	**680-760**	**380-540**

Table 2. Tocols (17) and Carotenoids (18) content of RPO, PO and PS.

Palm oil is naturally rich of beneficial minor components, in particular tocotrienols, tocopherols and carotenoids (Table 2). Tocotrienols and tocopherols are vitamin E family members widely known for their health benefits. The vitamin E content in palm oil is unique because it is rich of tocotrienols rather than tocopherols (Table 2). Decades of research on tocotrienols, have shown that they have diverse biological activities, such as antioxidant, anti-inflammatory, anticancer, neuroprotective and skin protection actions; in addition, they improve cognition, bone health, longevity and reduce cholesterol levels in plasma (19).
Plant carotenoids are the primary dietary source of provitamin A worldwide. In developing country, where the vitamin A deficiency is one of the major health problem, moderate amounts of palm oil satisfy the requirements of this vitamin at low cost. In addition, RPO intake has been shown to be more effective than synthetic β-carotene supplementation to increase the maternal milk concentration of this compound (20). Beside the well-known functions of carotenoids and vitamin A in visual process and in the regulation of cell proliferation and differentiation, carotenoids have also a cardio-protective action (21).

CONCLUSIONS

This review shows that the massive attack against the use of palm oils in food production is largely unjustified. In fact,

within the limits of a balanced diet and according with the forthcoming EU regulation, the consumption of palm oil should not expose to more health risk than the other fats usually employed in the food industries. Moreover, palm oil is not only rich in SFAs, but also in carotenoids and tocotrienols that can counteract the potential negative effects of other components and also can provide several additional health benefits. Further studies are needed to better clarify the contribution of palm oils to human health.

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NanoPack:
State-of-the-art packaging to improve food safety and reduce food waste

KEYWORDS: Nanotechnology, active packaging, food safety, innovation.

Abstract NanoPack is an exciting new Horizon-2020 funded project aiming to develop and demonstrate antimicrobial food packaging films based on natural nanomaterials to extend the shelf-life of perishable foods. Harnessing the power of nanotechnology, natural Halloysite Nanotubes (HNTs) will be applied as reliable and safe carriers of bioactive compounds. These will be dispersed in polymers, to produce plastic films which will exhibit a broad spectrum antimicrobial activity, unmet by existing state-of-the-art materials. The resulting films will be used to develop active packaging for use across a range of food products. The project will develop, scale up and run pilot lines in operational industrial environments to manufacture antimicrobial films that are commercially feasible and accepted by retailers and consumers alike.

WHY THIS PROJECT

Food spoilage, caused by microbial growth, is a major cause of both food losses and foodborne illness. In Europe, despite being the global region with the lowest burden from foodborne illness, almost 3000 deaths per year are caused by preventable diarrheal foodborne diseases caused by contaminated food (1).

At the same time, an estimated 1.3 billion tonnes, or 32% of all food produced globally, are lost or wasted each year (2). This also represents a massive waste of the valuable resources used in food production (water, energy, work, money), and CO_2 emission produced from food waste rotting in landfills, which contributes towards climate change.

Food safety and food waste are therefore high priority issues for global and EU organisations, as evidenced by initiatives like the World Health Organization's strategic plan for food safety 2013-2022 and the European Parliament's proposal to reduce food waste by 50% before 2030 (3, 4).

Innovative food packaging is a promising solution for addressing these issues. Nanotechnology in particular has been identified by the European Commission as one of six key enabling technologies (KET) that have a wide range of product applications and can provide the basis for innovation across industrial sectors (5). NanoPack aims to harness the potential of nanotechnology to develop antimicrobial polymer films for use in active packaging applications, to slow down food spoilage and extend the shelf life of food products, ultimately contributing to the goals of improving food safety and reducing food waste.

BACKGROUND – STATE OF THE ART AND INNOVATION BARRIERS

To date, antimicrobial food packaging materials have not been used on a large scale. This is in part due to concerns about the safety and toxicity of other commonly-used antimicrobial agents, for example silver or silver-based compounds, but also since their efficacy generally relies on direct contact with the microbes (6). Their use is also limited by their high price and their adverse effect on packaging materials, affecting for example colour, transparency, oxygen and/or water vapour permeability.

One of the main barriers to innovation implementation in the European food technology sector is cost. Considering that 99% of EU food companies are small or medium size enterprises (SMEs), uptake of new technologies requiring change or upgrade of existing production machinery is challenging. NanoPack aims to lower these barriers to innovation implementation.

HALLOYSITE NANOTUBES – AFFORDABLE AND AVAILABLE

NanoPack will use Halloysite nanotubes (HNTs) as carriers for the encapsulation and controlled release of antimicrobial

compounds from the active food packaging films. HNTs are considered to be one of the most promising natural nanomaterials due to their unique combination of properties, including hollow tubular structure, large aspect ratio, low cost and abundant natural availability, good biocompatibility, and high mechanical strength. Table 1 compares the properties of HNTs to synthetic carbon nanotubes (CNTs), which are among the most studied nanomaterials to date. Clearly, HNTs offer unique advantages for applications requiring the usage of safe, non-toxic and environmentally friendly nanocapsules. Being a natural resource, HNTs do not demand extensive manufacturing efforts inevitably required to produce their synthetic counterparts. The HNTs low price combined with their abundant deposits around the world make them more suitable for mass-scale industrial applications than CNTs. However, HNTs are still not fully explored, and their immense potential to revolutionise the field of active polymer nanocomposites remains unrealized.

	Halloysite nanotubes	Carbon nanotubes
Dimensions	15 nm (diameter) 1-3 µm (length)	2-10 nm (diameter) 1 µm (length)
Source	Natural mineral	Synthetic
Availability	Thousands of tons	Grams to kilograms
Biocompatibility	Biocompatible	Toxic
Price	~4-10€ per kg	~300€ per kg

Table 1. Comparison of the properties of Halloysite nanotubes to carbon nanotubes.

ESSENTIAL OILS – NATURAL AND VOLATILE ANTIMICROBIALS

The emergence of antibiotic resistance has led to renewed interest in exploring the potential of plant-derived antimicrobials as an alternative strategy to combat microbial infections. NanoPack will harness the antimicrobial potential of plant-derived essential oils, which have demonstrated potent, antimicrobial activity against a wide range of microorganisms including bacteria, yeast and moulds, even at very low concentrations. They are commercially available at low cost and generally recognised as safe by the American Food and Drug Administration (FDA) and categorized as flavourings by the European Decision 2002/113/EC (22). The volatility of essential oils has so far proved a major challenge for incorporating them into polymers using conventional compounding and processing techniques.

TOWARDS THE NANOPACK CONCEPT

While encapsulation of antimicrobial compounds within nanomaterials on a large scale has so far proven a significant challenge, lab-scale studies have demonstrated the use of HNTs as nano-scale containers for the encapsulation of biologically active molecules.

For example, HNTs have been successfully used to encapsulate carvacrol and carvacrol/thymol mixtures. Preliminary results have shown that encapsulating carvacrol within HNTs increases its thermal stability range by more than 40 °C (7), minimizing the loss of the highly volatile essential oil during high-temperature polymer processing. This enables melt compounding of HNTs-essential oil nanocapsules with different polymers, and subsequent film production, although so far this has only been demonstrated using a semi-industrial scale lines. The resulting active polymer films, shown in Figure 1, exhibit effective broad spectrum antimicrobial activity against a range of bacteria and fungal moulds. Their potential for reducing decay development and bacterial growth, and as a result increasing shelf-life, has been demonstrated in both model and real food packaging systems on a range of different food products (8, 9). Unlike common antimicrobial agents that function only in direct contact with packaged food, in the NanoPack system, essential oils are released, in a controlled manner, as vapour from the packaging materials into its headspace, and are capable of sanitizing both the product surface and the headspace.

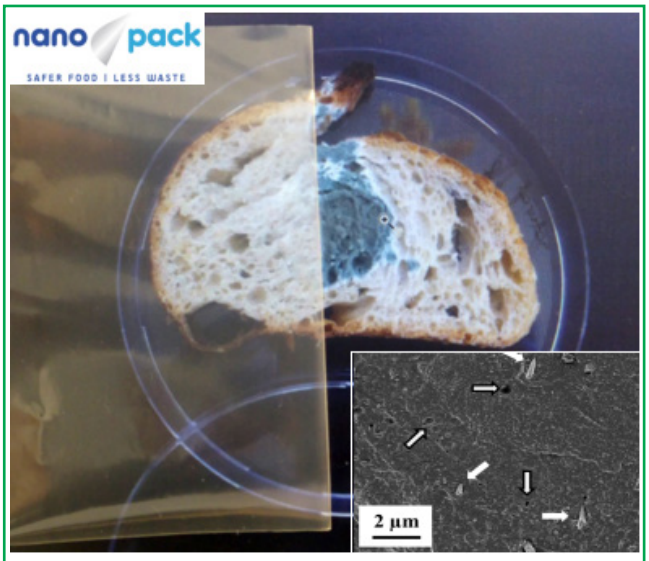

Figure 1. NanoPacks antimicrobial polymer films reduce decay development and fungal growth in bread, thus enhancing its shelf life. The inset shows high-resolution scanning electron micrograph of the active film nanostructure, highlighting the fine distribution of the loaded HNTs nanocapsules within the polymer matrix.

To successfully apply this innovative technology on a mass industrial scale, the NanoPack project has developed a methodology based on four pillars: product readiness, pilot testing, assessment and impact generation.

NANOPACK METHODOLOGY: FOUR PILLARS TO BREAKING THE INNOVATION BARRIER

Pillar 1: Product readiness

The first phase of the project will focus on optimizing a three-part process to ensure product readiness:

- In a first stage, the HNTs will undergo chemical modification to maximize their essential oil loading potential and subsequent controlled release from the HNTs-essential oil nanocapsules over time.
- The second stage will involve testing of synergic combinations of essentials oils to achieve the highest antimicrobial efficacy at low concentrations, to minimize undesired organoleptic (sensory) effects on the final packaged food product. Processes for loading of the essential oils onto HNTs will be optimized in order to achieve high loading concentrations.
- The processes for producing loaded-HNTs/ polymer nanocomposites by melt compounding and subsequent high-quality film production, while maintaining antimicrobial bioactivity will be investigated.

Pillar 2: Pilot testing
The second major phase of the NanoPack concept involves ensuring scalability of all production steps and carrying out pilot tests of all packaging production stages on existing pilot production lines. First, the methods for HNTs surface functionalization and production of HNTs-essential oil nanocapsules will be optimized for large scale production. Once these challenges have been surmounted, the project will carry out pilot line production of master batches of the loaded-HNTs/polymer nanocomposites, using on a variety of antimicrobial essential oils.

Active, polymer/HNTs nanocomposites will be further processed into prototype flexible food packaging film products using existing pilot line facilities in both large and SME industrial settings. The antimicrobial properties of these new films, as food packaging, will be demonstrated using three categories of food products: fresh meat, bakery and dairy. All pilot line testing will employ existing small and large production lines, to ultimately enable seamless and cost-effective adoption of the novel packaging production in industrial settings.

Pillar 3: Assessment
NanoPack has a strong focus on assessment of the developed product from all angles. The results from the pilot testing will be quantitatively and qualitatively assessed and validated. A comprehensive investigation into the quality of the HNTs-essential oil nanocapsules and polymer/HNTs nanocomposites (e.g. mechanical, rheological, optical, thermal, structural properties) and the resulting packaging films (e.g. physical and processing parameters) will be carried out. Food products packaged in NanoPack films/trays will undergo shelf-life assessment (including investigation of bacterial growth and organoleptic properties).

Concern about impacts on health and the environment is a potential barrier limiting widespread use of nanotechnologies in food packaging. As such, health (including occupational) and environmental safety assessment of NanoPack products and processes is prioritized. Assessment activities include migration studies, investigation of the toxicological and ecotoxiological profiles of HNTs, exposure assessment during production and product application, and human and environmental risk assessment. Life Cycle Assessment will be carried out to determine the environmental impact of all stages of NanoPack materials production and waste management when applied on an industrial scale. The social and economic costs and benefits of the NanoPack system will be performed through Life Cost Cycle Analysis, to identify the capital and operational costs, and Social Life Cycle Assessment to evaluate socio-economic impacts.

Pillar 4: Impact generation
Current penetration of active and intelligent packaging in the European market is limited by lack of end-user acceptance due to conservativism all along the EU food chain regarding innovations in food. There is also a history of consumer rejection of useful innovations often linked to insufficient communication. To minimize the likelihood of consumer rejection and maximise commercialisation potential, NanoPack will conduct consumer and retailer acceptance studies. Results will feed directly into the project's dissemination activities including the development of recommendations on how to communicate and position novel food packaging products to promote adoption by consumer and retailers. A detailed exploitation strategy will include a plan for taking each product or process developed by NanoPack to market readiness, with a focus on technology transfer and fast commercialisation of NanoPack products across the supply chain.

IMPACTS OF THE PROJECT

NanoPack is expected contribute to the challenge of providing sustainable solutions for enhancing food safety for consumers by inhibiting growth of microbes in food products, which will prevent early spoilage and foodborne illness outbreaks. Furthermore, extending the shelf-life of food products will help reduce the massive 1.3 billion tonnes of food wasted per year. Minimizing food waste caused by spoilage can reduce operational costs related to wastage for food manufacturers, packagers, and retailers, in turn potentially lowering food costs for consumers.

NanoPack will organize training workshops to ensure technology transfer to the industrial and scientific community and to build an educated workforce and enable manufacturers across the supply chain (including SMEs) to take advantage of the technology developed during the project. This will contribute towards position Europe as a global leader in food nanotechnology and smart antimicrobial packaging, thereby increasing competitiveness and growth of the industry.

CONSORTIUM

NanoPack is coordinated by Prof. Ester Segal, of the Technion – Israel Institute of Technology. Eighteen partner organisations from 11 countries, bring together competences from nanotechnology, food packaging manufacturing/testing, food production, life cycle assessment, food regulation and safety assessment, consumer research and communication.

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NEWS NEWS NEWS NEWS NEWS NEWS NEWS

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The food quality labels:
Awareness and knowledge of Slovenian consumers

KEYWORDS: Food quality labels, food labelling, awareness, knowledge, Slovenia.

Abstract Previous studies have rather neglected the issue of consumer knowledge of food quality labels. This paper aims to discover the awareness and knowledge of food quality labels most frequently appearing on the Slovenian food market. On a representative sample of Slovenian consumers (N= 650), a self-administrative electronic questionnaire was compiled. Survey findings reveal that Slovenian consumers show little awareness of EU food quality labels. Higher awareness of national labels was observed. Consumers with higher education and income levels show a higher level of knowledge of national labels for protected designation of origin (national PDO), as compared to others. Survey findings show that promotion of EU labels should intensify to achieve higher consumer awareness and knowledge.

INTRODUCTION

In recent years, consumers in the European Union (EU) are more and more critical in their food choices, showing an increased interest in quality differentiation and quality labelling (1-4). Food quality labelling has been introduced by different entities, policy makers and private food companies (2) so as to guarantee the quality of products in terms of composition, origin or method of production, and to help consumers choose quality food (5, 6).
Slovenian consumers are provided with numerous EU and national food labels designating the origin and quality of food products. In 2002, Slovenia developed an umbrella national scheme, a collection of food labels of different colours and of EU-label-like characteristics (7). Formerly, Slovenian food producers were required to produce a national registration for PDO, PGI or TSG and thereafter, an EU registration.
As from 4 January 2016, the EU labelling of food products with a PDO or a PGI label has been mandatory (8). The EU Protected designation of origin (PDO) label and its national Slovenian equivalent (national PDO label) cover the agricultural products and foodstuffs, which are produced, processed and prepared in a given geographical area, using the recognised traditional know-how.
The EU *Protected geographical indication* (PGI) label and its national Slovenian equivalent (national PGI) indicate a link with the geographical area at least at one of the stages of production, processing or preparation, and the EU *Traditional Speciality Guaranteed* (TSG) label and its Slovenian equivalent (national TSG) highlight the traditional character of a product (9-12). To date, eight Slovenian food products with an EU and a national PDO label, twelve food products with an EU and a national PGI label, and three food products with an EU and national TSG label have been registered (12).
The EU introduced the EU organic logo, which highlights that at least 95 % of agricultural ingredients are organic (13).
In 2015, 3,417 farms (4.7 % of all farms in Slovenia) practised organic farming in Slovenia, and 79 % thereof obtained certification (14).
In June 2016, the Slovenian authorities started the registration of specifications of Selected Quality – Slovenia for milk and dairy products, and beef and poultry meat produced according to the registered specifications (15).
Consumer awareness and understanding of label information are crucial for determining, maintaining, and communicating food quality labels (16-17).
The main aim of this paper is to discover the awareness and knowledge of quality labels appearing on the Slovenian food market. The review of 35 published researches on how EU quality labels affect consumers shows that quality labels can have the function only to the extent that consumers are aware of them, understand them and use them in their decision-making (18). Past research shows that interpretation of the EU label contents is rather perplexing and bewildering to Slovenian consumers (1, 10, 19-20). Analysis of European consumer awareness and determinants of use of PDO, PGI and TSG labels confirmed low awareness among the consumers living in the Northern countries, and higher awareness among consumers living in the Southern countries as a result of a long-standing EU quality label tradition (19-21). However, studies to delve into the kind of knowledge the consumers possess of quality labels, are still outstanding.
Our study hypothesizes that Slovenian consumers possess a higher level of awareness and knowledge of national quality labels than of EU quality labels, on account of less experience with EU labels (19-24).

MATERIALS AND METHODS

For the purposes of this study, a representative sample (online) of Slovenian consumers as to age, gender, education was

prepared (see, Table 1). The total of 650 consumers participated, of which 316 (48.6%) females and 334 (51.4%) males. Age of the respondents ranged from 18 to 65 years, i.e. 12.5% aged 18-25 years, 20.6% aged 26-35 years, 23.1% aged 36-45 years, 21.7% aged 46-55 years, and 22.1% aged 56-65 years. One-third of respondents were educated to graduate (34.3%) and postgraduate level (5.1%); 11.2% were educated to high school, 38.5% to secondary school and 8.6% to technical school level, and 2.3% completed primary school education or less. A salary of less than 1000 net Euros per month was received by 19.4% of respondents, between 1001 and 2000 Euros was received by 32.8% of respondents, between 2001 and 3000 Euros by 20.2% of respondents, and over 3000 Euros by 8.8% of respondents.

Respondents were asked to complete a self-administrative structured electronic questionnaire in December 2016. The questionnaire was divided into three parts. The first part sought answers to the question of understanding and awareness of six most frequently used labels (EU PDO, national PDO, EU PGI, national PGI, EU organic, and national Selected Quality - Slovenia) – see Table 2. The second part analysed the consumer knowledge of labels. The third part of the questionnaire focused on socio-demographic information of respondents, including their age, gender, level of education, and level of income.
Two pre-tests with 10 professionals and 10 consumers were conducted to ensure the survey was worded appropriately for cultural and professional term considerations. To establish content validity of this survey instrument, a panel of consumers reviewed the draft proposed questions and provided feedback to the authors, to enhance the intelligibility of the questionnaire. Minor revisions were done prior to survey. Data were coded and analysed by SPSS 24.0, using descriptive statistics. Chi-square (χ^2) tests were used to determine significant differences in consumer knowledge of quality label according to their demographic attributes, such as gender, age, income, and education. The reliability of the questionnaire was tested using Cronbach's coefficient alpha, which was 0.86 and indicates that the measuring instrument is sufficiently reliable.

RESULTS AND DISCUSSION

Table 2 shows that around 65% of respondents reported the awareness of two national Slovenian quality labels (national PDO and PGI), whilst only 30.15% and 36.62% reported the awareness of EU labels – EU PDO and EU PGI, respectively. The EU organic label was well-known to 44% respondents. A higher awareness of the EU organic label, as compared to the EU PDO / EU PGI labels, may be due to the fact that there are many more food products with an EU organic label, than products with an EU PDO / EU PGI label on the Slovenian market (14).

Characteristics	Frequencies	Percentage (%)	Characteristics	Frequencies	Percentage (%)
Gender			*Education*		
Male	334	51.4	Primary school or less	15	2,3
Female	316	48.6	Technical school	56	8,6
Age			Secondary school	250	38.5
18-25	81	12.5	High school	73	11.2
26-35	134	20.6	University degree	223	34,3
36-45	150	23.1	Postgraduate degree	33	5,1
46-55	141	21.7	*Family income*		
56-65	144	22.1	< 1000 €	126	19.4
			1.001 € - 2.000 €	231	32.8
			2.001€ - 3.000 €	131	20.2
			Over 3.000 €	57	8.8
			Do not want to answer	123	18.9

Table 1. Socio-demographic profile of surveys (n=650).

Food quality labels	Description of food quality labels	Example of product with food quality label
označba porekla | Republika Slovenija Ministrstvo za kmetijstvo, gozdarstvo in prehrano	National PDO	
geografska označba | Republika Slovenija Ministrstvo za kmetijstvo, gozdarstvo in prehrano	National PGI	
IZBRANA KAKOVOST Slovenija	National Selected quality	
PROTECTED DESIGNATION OF ORIGIN	EU PDO	 
PROTECTED GEOGRAPHICAL INDICATION	EU PGI	
	EU Organic	

Table 2. Food quality labels used in Slovenia.

EU PDO	30.15	National PDO	65.38
EU PGI	36.62	National PGI	64.15
EU Organic	44.00	National Selected Quality	40.31

Table 2. Consumer awareness of different quality labels (valid %).

Findings as to the knowledge of quality labels are similar to those of the awareness of quality labels. Lower percentages were found, as shown in Table 3.

Difference in the knowledge of Slovenian labels, and their EU equivalents, may be explained by the fact that Slovenian food products gained national registration five or more years earlier, than the EU registration, due to a complicated and costly EU procedure (24).

An intensive information and promotion campaign that the Slovenian Ministry of Agriculture, Forestry and Food (MAFF) launched in 2007 was intended for national quality labels only. Slovenian consumers showed better knowledge of the Slovenian labels as they had longer been familiar with them, than with the European ones.

These findings confirm the hypothesis that Slovenian consumers are more familiar with our national quality labels, than with their EU equivalents (23). Relatively poor awareness of the national label *Selected Quality - Slovenia* may be due to its recentness on the Slovenian market, as it was introduced only in 2016.

EU PDO	14.46	National PDO	48.62
EU PGI	19.69	National PGI	47.54
EU Organic	25.38	National Selected Quality	17.85

Table 3. Consumer knowledge of different quality labels (valid %).

Table 4 shows significant differences observed when considering the knowledge of the national quality label PDO in relation to education and income level. Respondents with higher education (χ^2 = 18.36, p= 0.02) and higher income levels (χ^2 = 11.35, p= 0.002) are significantly more likely to have better knowledge of national PDO, than other respondents. This finding may be interpreted by the fact that most Slovenian consumers with higher education and higher income levels willingly improve their knowledge of alimentary traditions and thus show greater interest in food of Slovenian origin, as compared to others with lower education and income levels (25).

There are significant differences between respondents, based on age level, in the knowledge of national PGI. Respondents, who were more advanced in age (χ^2 = 10.61, p= 0.03), showed better knowledge of national PGI, than the younger ones. This may be explained by the fact that the older generations are more attached to the food of Slovenian origin, as compared to younger generations (11).

	Gender		Age		Education		Income level	
	Pearson chi-square	Sig.	Pearson chi-square	Sig.	Pearson chi-square	Sig.	Pearson chi-square	Sig.
National PDO	0.06	0.80	5.58	0.23	18.36	0.02*	11.35	0.002**
National PGI	0.18	0.67	10.61	0.03*	2.58	0.76	7.33	0.12
National Selected quality	0.22	0.63	4.58	0.33	2.15	0.82	0.83	0.93
EU PDO	0.03	0.87	1.84	0.76	5.33	0.38	1.68	0.79
EU PGI	1.23	0.27	0.34	0.99	7.75	0.17	3.74	0.44
EU Organic	0.15	0.70	0.55	0.97	2.61	0.76	3.47	0.48

Chi-square test

Table 4. Consumer knowledge of quality label by gender, age, education and income level

CONCLUSIONS

The study findings reveal that Slovenian consumers show little awareness of food quality labels and frequently misinterpret the real meaning of labels observed. However, higher awareness of national labels only was observed. Consumers with higher education and higher income levels were significantly more likely to possess better knowledge of national PDO, than other consumers. Respondents, advanced in age, showed better knowledge of national PGI, than younger and older ones.

We established that Slovenian consumers were much less aware of EU labels than consumers from Southern countries, such as French, Italian and Spanish consumers, and similarly aware as consumers from Northern countries, such as Belgian, Norwegian and Polish consumers, due to less experience with the EU labels.

The study findings expose the need of expanding and intensifying the promotion and communication activities towards EU quality labels, so as to improve consumer awareness and knowledge thereof.

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PEER REVIEWED

Identification of products attributes important for food choice

KEYWORDS: Consumers, food product development, quality, safety.

Abstract The aim of this paper was to identify product attributes important for young consumers' in food choice, as data useful for producers in food product presentation and commercialisation on target market. Consumers' attitudes and importance of food products quality and safety information were identified and analysed regarding observed population of 720 younger consumers, and crossing the groups formed based on age and sex. Consumers' interest in new food products, some specific quality and safety indicators and packaging attributes were identified and their importance for food choice compared. Product attributes, important for attracting consumer attention were identified and ranked: food safety, nutritional and sensory quality, shelf life, price, trademark, packaging design, and origin.

INTRODUCTION

The significant changes in life-style impact on consumers' food choice. They are more and better informed, with higher demands and individual standards. Assortment of food products offered on the market and competitiveness affect the business success. Small and Medium Enterprises (SMEs) are contributors to growth and employment in the European Union, usually offering products for regionally limited market and facing with the challenge of being competitive (1). Consumer's product acceptance is crucial for product success and survival in the market, but it is changing over time (2). Producers should recognise or uncover consumers' expectations on target market and use them as ideas for food products improvement and development (1, 3). Multiple factors as consumer gender, age, level of education, food-related advertising, package design, food product nutritional quality and sensory properties or price affect on food product consumption (4-6).
Nutritive and sensory quality characteristics or food-related emotions impact on food acceptance, pleasure in consumption and selling (7). However, customer should perceive the product on shelf, in order to consider these elements. The food product packaging design may attract consumer's attention with contemporary aesthetic elements, sharp and colours (8). Various packaging materials and technologies are available in the food industry, they are continuously improved and updated to meet changing consumer demands, and impact on purchasing decision and consumption (1, 4). Food products package or labels contain important information about food quality, nutritional value and safety (9-12). It includes data on ingredients, nutrients, allergens, additives, product quantity, processing method, storage conditions and shelf life (13-15). Food safety is imperative in contemporary food processing today, and includes row materials, processing and packaged product storage, employees' hygienic behaviour and clean facilities (16-19).

Selected food product quality parameters and labelled data should be highlighted on packaging and emphasized in advertising to attract consumers' attention and contribute to product positioning in the market (20, 21).
The aim of this paper was to identify product attributes important for young consumers' in food choice, as data useful for producers in food product presentation and commercialisation on target market. Consumers' attitudes and importance of food products quality and safety information will be examined and analysed regarding total observed population, and crossing the groups of consumers formed based on age and sex.

METHODOLOGY

Young adults, who attend high school or undergraduate study, are considered as new food products consumers. Respondents were selected from three bigger regional centres in the Republic of Srpska (Bosnia and Herzegovina) with public educational institutions, as follows: **adolescents** (students in high school in 3 towns); and **young adults** (students from undergraduate study in 5 towns).
Planned research was conducted with 720 young consumers, using self reported data in affective sensory tests. Data were analyzed regarding total observed population (1); and crossing the groups of consumers formed based on: (2) age; (3) sex.
The first classification was related to the grouping based on age: (i) 360 **adolescents** (**P-group**)16-18 years old, boys average age 16.72 ± 0.64 years and girls 16.73 ± 0.63 years; and 360 **young adults** (**S-group**) 20-25 years old, males average age 22.61 ± 2.27 years and 21.69 ± 1.41 years for women. The second classification was based on sex: (ii) 290 **males (M-group)** and 430 **females (F-group)**. Respondents' classification based on sex and age was made without possibility for further selection. Standard deviations within each group of respondents were calculated.

Statement scored on 3 points scale (Very important = 3; Important = 2; Not important = 1)		Mean ± SD[a]	Scores ranking[b]	Frequency of responses n_x [c] in % of all responses (N=713)		
				n_3	n_2	n_1
M1	Manufacturer is known in the market as a successful trademark.	1.97 ± 0.03	6	19.21	57.78	23.00
M2	Product has "domestic origin".	1.65 ± 0.07	8	15.43	32.82	51.75
M3	Product has a nice packaging design.	1.84 ± 0.03	7	17.67	48.81	33.52
M4	Product has adequate nutritional and sensory quality.	2.67 ± 0.08	3	70.55	26.65	2.66
M5	Product price is proportional to the quality.	2.35 ± 0.04	5	38.85	53.86	7.29
M6	Product is fresh, with longer shelf life than other similar products.	2.57 ± 0.06	4	62.41	32.96	4.63
M7	Product is prepared in hygienic conditions.	**2.80** ± 0.08	*1*	**83.73**	13.74	2.52
M8	Food product packaging is undamaged.	2.78 ± 0.09	2	82.33	14.31	3.22

Question offered with Yes/No answers		yes response[d]	
		n_{yes}	$\%_{yes}$
Q1	Would you buy the product made as a good copy of your favorite brand?	357	49.58
Q2	Do you believe in accuracy of the data in advertising statements?	135	18.75
Q3	Do you prefer to taste new food products?	597	83.03
Q4	Would you buy a product with reduced price because of expiry of durability?	137	19.05
Q5	Would you return purchased product, if you discover that it is not good?	602	83.72

Table 1. Statements that contain data which may be important in consumers' food choice, mean scores, frequency of responses; and questions with frequency of yes responses and % calculated based on all responses on each question.
[a] Mean ± SD = mean values for scoring on 3 points scale ± standard deviation for all responses (N=713),
[b] scores ranking; [c] frequency of responses in % (n_3 - Very important ; n_2 - Important; n_1 - Not important);
[d] Frequency of yes responses (n_{yes}) on each question *(Qx)* and $\%_{yes}$ calculated based on all responses (N=720).

A printed form questionnaire were used to obtain personal data, in first part (name of educational institution, secondary or higher level, study year, individual age; sex; economic status), and in second part were evaluated importance of 8 statements (1 = Not important; 2 = Important; 3 = Very important) and 5 questions with offered Yes/No answers that contain specific data which may be important in food choices (Table 1). The respondents spent approximately 10 minutes for answering.

Question	Compared groups and respondents total number (N)	Frequency of responses (n) on E1 question in group and within group					χ^2[a]	p[a]
		Z			L			
		(n)	(n_Z)	(%)	(n_L)	(%)		
E1	P-group	360	329	91.39	31	8.61		
	S-group	360	332	92.22	28	7.78		
	N= 720						0.166	0.684
	M-group	290	263	90.69	27	9.31		
	F-group	430	398	92.56	32	7.44		
	N= 720						0.804	0.370

Table 2. Answers (n) regarding respondents' individual economic status compared between the groups formed based on respondent's age and sex, and Chi-square Analysis.
E1 **=** How would you classify your economic status? (Z = satisfactory; L= low)
P = Adolescents; S = Young adults; M = Male; F = Female
[a] $\chi^2_{1;0.05} = 3.841$; p<0.05;

The results of the survey were grouped accordingly. The descriptive statistics included means and standard deviations (SD), frequencies (n; %) (Table 1). We stated hypothesis that there is no significant difference in the attitude toward specific information crossing the groups of consumers regarding: (1) age; (2) sex. All analyses were performed using Statistical software *3BStat* Gold Edition, Version 1.01. (22). Values of P below 0.05 were considered statistically significant. After answers analysis, the Statistical Chi-square Analysis and Z-test were performed and used for testing the hypothesis and the significance (p<0.05) of differences across the groups.

The **personal economic status of respondents** may impact on food choice. Analyse revealed homogeneous distribution in compared groups included in the research, as most respondents had satisfactory, and a few low economic status (Table 2). There were no conditions for sub-groups forming as difference between formed groups based on age ($\chi^2_{1;0.05} = 0.166$; p>0.05) and sex ($\chi^2_{1;0.05} = 0.804$; p>0.05) wasn't significant.

RESULTS AND DISCUSSION

The new product development for regionally limited market may be way for SMEs success. Adapting to the market changes requires product designing for real consumer. Considering that numerous factors affect on food choice and attract attention in the target market, it is necessary to identify consumer's **attitudes in product attributes** perception. The survey included consumer's individual evaluation of importance (on 3 points scale) for 8 selected statements, each indicating on products characteristics or attributes, and 5 questions (with offered Yes/No answers) that contain specific data which may affect the food choice.

Trademark, product origin and package

Customer should perceive product on shelf, in order to consider its attributes. Stronger trademarks invest more in marketing activities and easier acquire consumer's confidence in new products quality (23). Consumers pay more for known brand than for other similar products (24). The research indicated that **product's successful trademark** ***(M1)*** importance was lower ranked, on sixth place, between information evaluated in food choice ***(M1-M8)*** (Table 1). Between P- and S-group responses difference regarding **trademark** importance was significant ($\chi^2_{2;0.05} = 7.913$; p<0.05), but between M- and F-group (Table 3) wasn't ($\chi^2_{2;0.05} = 5.554$; p>0.05).

Consumers' trust in producers is associated with the general confidence in food quality and safety (18) and trademark (24). Analysis (Table 1) showed that half of respondents (49.65%) included in the survey **will buy a good copy of favourite brand product** (***Q1***), with more confirming answers (Table 4) in S- than P-group ($\chi^2_{1;0.05} = 28.008$; p<0,05) and in F- than M-group ($\chi^2_{1;0.05} = 4.710$; p<0.05).
The results indicated that domestic producers cannot rely only on consumers' loyalty, as **the importance of product domestic origin** *(**M2**)* had the lowest rank in food choice (Table 1), with similar value for compared groups formed based on age ($\chi^2_{2;0.05} = 2.228$; p>0.05), but it had less importance for females than males ($\chi^2_{2;0.05} = 12.955$; p<0.05) (Table 3).
The product package is attracting consumers' attention with its design, colour and shape. They are first visual product quality indicators in communication with consumers (2). Package appearance may be related to the product sensory and quality properties evaluation and expected price (25). Analysis showed that **packaging design** (***M3***) ranked on penultimate place based on importance scores (Table 1). The responses distribution (Table 3) were similar when compared based on consumer age ($\chi^2_{2;0.05} = 2.191$; p>0.05) and sex ($\chi^2_{2;0.05} = 2.004$; p>0.05).

Statement	Compared groups and respondents total number	Frequency of responses on statement importance in group (n) and within group (n_x)[a]				χ^b	p^b
	(N)	(n)	1 (n_1)	2 (n_2)	3 (n_3)		
(M1) Manufacturer is known in the market as a successful trademark.	P-group	353	88	186	79		
	S-group	360	76	226	58		
	N= 713					**7.913**	**0.019***
	Z-test[c]		1.211	**-2.726**	**2.124**		
	p[b]		0.226	**0.006***	**0.033***		
	M-group	286	64	155	67		
	F-group	427	100	257	70		
	N=713					5.5542	0.062
(M2) Product has "domestic origin".	P-group	353	185	108	60		
	S-group	360	184	126	50		
	N= 713					2.228	0.328
	M-group	286	135	90	61		
	F-group	427	234	144	49		
	N= 713					**12.955**	**0.002***
	Z-test[c]		**-1.990**	-0.628	**3.570**		
	p[b]		**0.047***	0.530	**0***		
(M3) Product has a nice packaging design.	P-group	353	109	179	65		
	S-group	360	130	169	61		
	N= 713					2.191	0.334
	M-group	286	93	148	45		
	F-group	427	146	200	81		
	N= 713					2.004	0.367
(M4) Product has adequate nutritional and sensory quality.	P-group	352	13	117	222		
	S-group	360	6	73	281		
	N= 713					**19.602**	**0***
	Z-test[c]		1.678	**3.909**	**-4.390**		
	p[b]		0.093	**0***	**0***		
	M-group	285	13	85	187		
	F-group	427	6	105	316		
	N= 713					**9.839**	**0.007***
	Z-test[c]		**2.560**	1.547	**-2.409**		
	p[b]		**0.010***	0.122	**0.016***		
(M5) Product price is proportional to the quality.	P-group	353	32	193	128		
	S-group	360	20	191	149		
	N= 713					4.303	0.116
	M-group	286	25	137	124		
	F-group	427	27	247	153		
	N= 713					**7.014**	**0.030***
	Z-test[c]		1.217	**-2.610**	**2.021**		
	p[b]		0.224	**0.009***	**0.043***		

Table 3. Responses on statements regarding products trademark (M1), origin (M2), package attractiveness (M3), product quality (M4) and its price relation (M5) importance in consumer food choice, compared grouped based on respondent's age and sex, Chi-square Analysis and Z-test
P = Adolescents; S = Young adults; M = Male; F = Female;
[a]1 = Not important; 2 = Important; 3 = Very important:
[b]$\chi^2_{2;0.05} = 5.991$; *p<0.05; [c]Z-test for proportions of two samples; *p<0.05;

Question	Compared groups and respondents total number	Frequency of responses on question in group (n) and within group (n_x)			χ^a	p^a
	(N)	(n)	yes (n_{yes})	no (n_{no})		
(Q1) Would you buy the product made as a good copy of your favorite brand?	P-group	360	143	217		
	S-group	360	214	146		
	N=720				**28.008**	**0***
	M-group	291	130	161		
	F-group	429	227	202		
	N=720				**4.710**	**0.030***
(Q2) Do you believe in accuracy of the data in advertising statements?	P-group	360	101	259		
	S-group	359	34	325		
	N=720				**40.709**	**0***
	M-group	290	70	220		
	F-group	429	65	364		
	N=720				**9.162**	**0.002***
(Q3) Do you prefer to taste new food products?	P-group	360	316	44		
	S-group	359	281	78		
	N= 719				**11.526**	**0.001***
	M-group	290	233	57		
	F-group	429	364	65		
	N= 719				2.491	0.114

Table 4. Answers on questions related to consumer's loyalty (Q1), trust (Q2), and preference to new food products (Q3), grouped based on respondent's age and sex, and Chi-square Analysis.
P = Adolescents; S = Young adults; M = Male; F = Female;
[a]$\chi^2_{1;0.05} = 3.841$; *p<0.05;

Product quality and price

Positive experience (26, 27), sensory properties, memory based expectations, attitudes and beliefs about product (2, 7) influence on food product actual perception. **The product's adequate nutritional and sensory quality *(M4)*** had high importance for most respondents included in the research and ranked on third place, after food safety indicators (Table 1). Grouped responses comparing reviled that there isn't significant difference in product's nutritional and sensory quality importance evaluation (Table 3) between young **adults** and adolescents ($\chi^2_{2;0.05} = 19.602$; p<0.05) or between females and males ($\chi^2_{2;0.05} = 9.839$; p<0.05), confirming importance of product taste and labelled data about ingredients and nutritional value (2).

Producers with known brand may set higher prices without adversely affect on their sales volumes (24). The emotional quality of product is advantage in the marketplace, if products are similar in quality and price (2, 25), while price is negatively correlated to demand (28). Research on the market indicated that consumers expect **proportional relation between product price and quality** (***M5***) (Table 1), without significant difference in evaluation ($\chi^2_{2;0.05} = 4.303$; p>0.05) between P- and S-group groups (Table 3), but with more importance for females than males ($\chi^2_{2;0.05} = 7.014$; p<0.05).

New products and consumers

Consumers obtain information about food quality and safety from family, friends, visual and written media or internet (14, 19). Marketing activities should link product quality characteristics with promotional activities to attract **consumers' interest in new products** (20), having in mind that health-conscious consumers will pay premium prices for special quality and healthful food (25). Consumer's interest in new products examination is reasonable and necessary activity before organizing product development. Encouraging is finding that 83.03% respondents (Table 1) **show intention to buy new food products** (***Q3***), where M- and F-group ($\chi^2_{1;0.05} = 2.491$; p>0.05) similarly responded, but more interest expressed S- than P-group ($\chi^2_{1;0.05} = 11.526$; p<0.05) (Table 4).

Food-related advertisements alone don't lead to increased food intake (5) and consumers prefer food products with quality guarantee (19) but advertising statements must be true. The most of respondents (Table 1) doesn't **believe in the advertising statements accuracy** (***Q1***) with variability in trust, less expressed in S- than P-group ($\chi^2_{1;0.05} = 40.709$; p<0.05) and in F- than M-group ($\chi^2_{1;0.05} = 9.162$; p<0.05) (Table 4). The results indicated on importance of building trust between producers and consumers, especially in advertising activities.

Food safety

Food safety is defined as condition of the foodstuffs in all stages of production, processing and distribution that guarantee protection of consumer's health. Examining respondent's knowledge about importance of **hygienic conditions in food manufacturing** ***(M7)*** showed that it is very high (Table 1) and the mean score ranked it on the first place of compared information importance for food choice. Analysis showed significant difference ($\chi^2_{2;0.05} = 13.532$; p<0.05) between P- and S-group and M- and F-group ($\chi^2_{2;0.05} = 21.513$; p<0.05) responses (Table 5). Manufactures should ensure product quality and safety in line with consumer expectations (29, 30). Also, **the packaging quality, shape and integrity** impact on product quality and safety and suggest its content characteristics (2).

Very high scored (Table 1) **importance of undamaged food product packaging** ***(M8)*** in food choice ranked on second place, with higher importance for young adults than adolescents ($\chi^2_{2;0.05} = 17.651$; p<0.05), and for females than males ($\chi^2_{2;0.05} = 22.431$; p<0.05) (Table 5).

Statement	Compared groups and respondents total number (N)	Frequency of responses on statement importance in group (n) and within group (n_x)[a] (n)	1 (n_1)	2 (n_2)	3 (n_3)	χ^b	p^b
(M6) Product is fresh, with longer shelf life than other similar products.	P-group	353	19	116	218		
	S-group	360	14	119	227		
	N= 713					0.909	0.635
	M-group	286	16	114	156		
	F-group	427	17	121	289		
	N= 713					**12.599**	**0.002**
	Z-test[c]		1.005	**3.208**	**-3.549**		
	p^b		0.315	**0.001***	**0***		
(M7) Product is prepared in hygienic conditions.	P-group	353	15	58	280		
	S-group	360	3	40	317		
	N= 713					**13.532**	**0.001**
	Z-test[c]		**2.907**	**2.063**	**-3.160**		
	p^b		**0.003***	**0.039***	**0***		
	M-group	286	14	53	219		
	F-group	427	4	45	378		
	N= 713					**21.513**	**0**
	Z-test[c]		**2.907**	**3.038**	**-4.238**		
	p^b		**0.004***	**0.002***	**0***		
(M8) Food product packaging is undamaged.	P-group	352	18	64	270		
	S-group	360	5	38	317		
	N= 713					**17.651**	**0**
	Z-test[c]		**2.810**	**2.904**	**-3.980**		
	p^b		**0.004***	**0.004***	**0***		
	M-group	286	16	57	213		
	F-group	426	7	45	374		
	N= 713					**22.431**	**0**
	Z-test[c]		**2.923**	**3.497**	**-4.579**		
	p^b		**0.003***	**0***	**0***		

Table 5. Responses on statements regarding food safety importance in consumer food choice (M6-M8), compared grouped based on respondent's age and sex
P = Adolescents; S = Young adults; M = Male; F = Female;
[a] 1 = Not important; 2 = Important; 3 = Very important
[b] $\chi^2_{2;0.05} = 5.991$; *p<0.05; [c]Z-test for proportions of two samples; *p<0.05;

Question	Compared groups and respondents total number (N)	Frequency of responses on question in group (n) and within group (n_x) (n)	yes (n_{yes})	no (n_{no})	χ^a	p^a
(Q4) Would you buy a product with reduced price because of expiry of durability?	P-group	360	64	296		
	S-group	359	73	286		
	N= 719				0.762	0.383
	M-group	290	68	222		
	F-group	429	69	360		
	N= 719				**6.084**	**0.014***
(Q5) Would you return purchased product, if you discover that it is not good?	P-group	360	312	48		
	S-group	359	290	69		
	N= 719				**4.572**	**0.033***
	M-group	290	239	51		
	F-group	429	363	66		
	N= 719				0.616	0.433

Table 6. Summary of answers on questions related to food safety knowledge (Q4) and habits (Q5), grouped based on respondent's age and sex, and Chi-square Analysis.
P = Adolescents; S = Young adults; M = Male; F = Female;
[a] $\chi^2_{1;0.05} = 3.841$; *p<0.05;

Food product labels contain information about food quality, nutritional value and safety (9, 12-15), including data on shelf life. **Consumers'** confirmed **familiarity with shelf life meaning and products freshness** ***(M6)*** (Table 1). It had similar meaning for P- and S-group ($\chi^2_{2;0.05}$ =0.909; p>0.05), but higher importance had for females than males ($\chi^2_{2;0.05} = 12.599$; p<0.05) (Table 5).

The shelf life is period of time when food product's safety, nutritional and sensory quality are guaranteed (9) and food should be consumed **before it expires.** Most of respondents (80.95%) confirmed knowledge regarding food safety (Table 1) **and that wouldn't buy products with reduced price because of expiry of durability** (***Q4***). The same value it has for P- and S-group ($\chi^2_{1;0.05} = 0,762$; p>0.05), but less females expressed willingness to buy so products than males ($\chi^2_{1;0.05} = 6,084$; p<0.05) (Table 6). **Consumers' awareness on food safety** impact on purchasing habits (19). Most of consumers (83.72%) expressed desire to protect their rights (Table 1) and intention to **return purchased product, if discover that it is not good** ***(Q5)***, with difference between P- and S-group answers ($\chi^2_{1;0.05} = 4.572$; p<0.05), but without difference ($\chi^2_{1;0.05} = 0.616$; p>0.05) between M- and F-group (Table 6).

CONCLUSIONS

Contemporary consumers are informed, have individual demands and standards. Younger consumer's interest in new food products, specific quality and safety indicators and packaging attributes were identified and their importance for food choice compared crossing the consumers groups formed based on age and sex. Some product attributes, important for attracting consumer attention were identified and ranked: food safety had the highest importance, followed by nutritional and sensory quality, shelf life, price, trademark, packaging design, and product origin. SMEs are usually offering products for regionally limited market, so it was important to identify attributes which attracts consumers attention in the first contact with the product, for user-oriented product presentation and commercialisation. As they are part of the information with impact on food choice, in the further research the consumers' interest in specific food products and health-related quality characteristics should be investigated and used in food product development.

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